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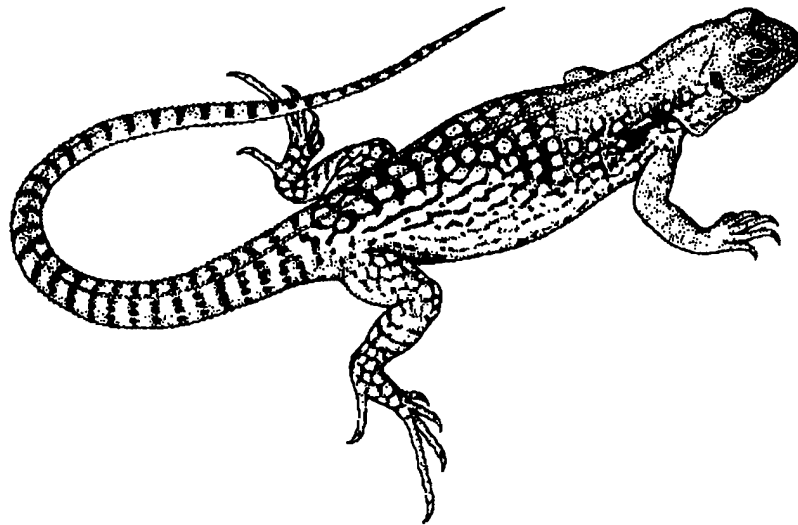
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THE ENERGETICS OF NON-SUSTAINABLE, BURST LOCOMOTION  
IN THE DESERT IGUANA, *DIPSOSAURUS DORSALIS*

by

Jeffrey M. Nedrow

B.S., United States Air Force Academy, 1993



A thesis submitted to the  
Faculty of the Graduate School of the  
University of Colorado in partial fulfillment  
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The Energetics of Non-sustainable, Burst Locomotion in the Desert Iguana,

*Dipsosaurus dorsalis*.

Thesis directed by Professor Todd T. Gleeson

Excess post-exercise, oxygen consumption (EPOC), once known as "oxygen debt," has been well documented in a wide variety of organisms. There are many factors implicated as responsible for EPOC. These include elevated lactate and hormone levels, depressed glycogen concentrations, increased body temperature (in endotherms), increased ventilation and heart rate, decreased phosphagen levels, and depressed oxygen concentrations in hemoglobin and myoglobin. Both the intensity and duration of exercise have been found to influence the length and magnitude of EPOC. Most of the previous research done on EPOC has focused on long duration, sustainable locomotion. For many animals, exercise of short duration is more ecologically relevant, yet little is known about EPOC under these conditions. There were three purposes for this study. First, I have focused on determining the influence of exercise duration on EPOC for short, ( $\leq 60$  seconds) maximal burst locomotion in the desert iguana, *Dipsosaurus dorsalis*. I have found that the length and magnitude of EPOC is positively affected by sprint duration. Secondly, I investigated the relationship between the adrenal hormones, epinephrine and norepinephrine, and EPOC. These hormones are known to elevate recovery metabolism and previous research has revealed that these hormones are increased in *D. dorsalis* following five minutes of treadmill exercise. I have found that administration of the  $\alpha$ -blocker, phentolamine, and the  $\beta$ -blocker, propranolol, prior to treadmill exercise significantly reduces EPOC duration and magnitude below saline injected control values. A third purpose of this research was to establish the energetic cost of non-sustainable transport,  $C_{ns}$ , for these animals. The cost of transport,  $C_t$ , has traditionally been measured for long duration, sustainable

exercise. EPOC has not been included in the calculation of the cost of sustainable locomotion because it constitutes such a small percentage of the total volume of oxygen consumed during exercise of long duration. My data have shown the volume of EPOC is greater than 90% of the total volume of oxygen consumed during sprints less than 60 seconds in length. It is my contention that the EPOC volume should be included in the cost of non-sustainable locomotion.

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## CHAPTER 1:

### EXCESS POST-EXERCISE OXYGEN CONSUMPTION: A REVIEW

#### Introduction

Whole animal metabolic rate is a measure of the rate of energy expenditure of an organism. There are a number of factors which have effects on an organism's metabolic rate. These include body temperature, body condition, age, body mass, hormone levels, environmental conditions, time of day, food and oxygen availability, developmental stage, and level of activity (Barnard and Foss, 1969; Gladden *et al.*, 1982; Heglund and Taylor, 1988; Baudinette, 1991; Withers, 1992; Swoap *et al.*, 1993). Of these, an animal's level of activity has some of the most profound effects on metabolic rate. An increase in the activity level of an animal can elicit a large increase in metabolic rate. Locomotion is a major component of an animal's activity level. Numerous studies have looked at the effects of locomotion on metabolic rate (Taylor *et al.*, 1970, Taylor, 1973; Taylor *et al.*, 1982, John-Alder *et al.*, 1983; John-Alder *et al.*, 1986; Heglund and Taylor, 1988; Baudinette 1991). These studies have found the general trend that as organisms exercise, oxygen consumption, ( $V_{O_2}$ ), increases and then declines back to resting values following the termination of exercise. My study will use measurements of  $V_{O_2}$  as a measure of metabolic rate much as these previous studies have done.

#### The Oxygen Debt Hypothesis

Concomitant with an increase in activity, animals exhibit an increase in oxygen consumption. However, following the cessation of exercise, oxygen consumption

does not immediately return to pre-exercise resting levels. This phenomenon was observed in man by Benedict and Carpenter (1910). They saw a 12-23% increase in  $\dot{V}O_2$  of two subjects during sleep seven hours after intense work. Numerous other studies have found similar results in that  $\dot{V}O_2$  can remain elevated for many hours following the termination of exercise (Gasser and Brooks, 1984; Gore and Withers, 1990a; 1990b; Bahr, 1992). The early work of A.V. Hill and associates attempted to determine the mechanisms of the increased metabolic rate following exercise. Hill (1914) found that during muscular contractions lactate was formed in the muscles. He also found that after the contractions ceased, glycogen replaced lactate "less a quantity which, calculated from oxygen consumption, had been lost by oxidation" (Hill, 1914). Hill and Lupton (1923) coined the term "oxygen debt" to describe the phenomenon of increased oxygen consumption following exercise. They defined oxygen debt as the "total amount of oxygen used, after cessation of exercise in recovery therefrom" (Hill and Lupton, 1923). Their " $O_2$  debt" hypothesis stated the oxygen debt was a result of the repayment of the oxygen deficit created during the transition from rest to exercise (Hill and Lupton, 1923). The oxygen deficit is produced because oxygen cannot be supplied quickly enough at the beginning of exercise to the working muscles to support the ATP demands of those working muscles. This causes a reliance on oxygen stores in the hemoglobin and myoglobin, cellular stores of phosphagens (creatine phosphate and adenosine triphosphate (ATP)), and glycolysis (Gatten, Jr., *et al.*, 1992). The oxygen and phosphagen stores are quickly depleted and then anaerobic metabolism is used to supply the ATP demands of the muscles until heart rate and ventilation increase sufficiently to meet the oxygen demands of the muscles. The use of anaerobic metabolism results in the production of lactate in the muscles. The excess lactate is either oxidized or used as a substrate for glycogen resynthesis. The energetic cost associated with the removal of the lactate formed during the  $O_2$  deficit was originally thought to be a major component of the  $O_2$  debt hypothesis (Hill and Lupton, 1923).

However, in recent years the oxygen debt hypothesis has been challenged by numerous authors (Gaesser and Brooks, 1984; Cerretelli, 1990; Bahr, 1992; Gatten, Jr., *et al.*, 1992). Studies have shown that lactate removal and oxygen consumption are not closely related (Bennett and Licht, 1973; Gaesser and Brooks, 1984; Gleeson and Dalessio, 1989; Wagner and Gleeson, 1996). Therefore, "oxygen debt" may be a misnomer because it implies a cause and effect relationship between lactate concentration and the elevation of  $\dot{V}O_2$ . However, ATP cost of lactate removal through glycogen resynthesis is greater than the ATP obtained by glycogen breakdown to lactate (Cerretelli, 1990). In other words, the volume of oxygen deficit should not be equal to the volume of oxygen debt. The proposed hypothesis of repaying the "oxygen deficit" incurred at the beginning of strenuous exercise with the elevated levels of oxygen consumption during "oxygen debt" after exercise should not be expected. Therefore, it seems that the classical  $O_2$  debt hypothesis is too simplistic of an explanation to describe the phenomenon of increased  $\dot{V}O_2$  following exercise. The relationship between lactate and oxygen consumption will be examined further in chapter 4.

#### Excess Post-exercise Oxygen Consumption

In 1984, Gaesser and Brooks offered the term excess post-exercise oxygen consumption, EPOC, to "avoid the implication of causality in describing the elevation of metabolic rate above resting levels following exercise." This thesis will use the terms EPOC and recovery oxygen consumption to describe the increase in  $\dot{V}O_2$  seen following exercise. Studies that have tried to identify the mechanisms responsible for EPOC have indicated that EPOC may be due to a variety of physiological factors including restoration of phosphagen levels (Bahr 1992; Gatten, Jr., *et al.*, 1992), increased ventilation and heart rate (Bahr 1992), removal of lactate and glycogen replenishment (Hill and Lupton, 1923; Brooks and Gaesser, 1980; Åstrand *et al.*, 1986), restoration of oxygen levels in hemoglobin and myoglobin (Bangsbo *et al.*,

1990, Gatten, Jr., *et al.*, 1992), increased body temperature in endotherms (Brooks *et al.*, 1971, Hagberg *et al.*, 1980; Gore and Withers, 1990a), and increased catecholamine levels (Barnard and Foss, 1969). For example, Gladden *et al.* (1982) have shown that norepinephrine increases post-exercise canine skeletal muscle  $\text{VO}_2$ . Catecholamines also have been shown to stimulate hepatic glycogenolysis and hepatic gluconeogenesis in some vertebrates (Kraus-Friedman, 1984; Mommsen *et al.*, 1988; Danulat and Mommsen, 1990). These actions by catecholamines may explain some of its effects on EPOC. The potential effects of the catecholamines on EPOC will be investigated further in Chapter 5.

Exercise duration and intensity are also known effectors of EPOC. EPOC is directly related to duration at intensities above 50%  $\text{VO}_{2\text{max}}$  (Gaesser and Brooks, 1984; Gore and Withers, 1990a ; 1990b; Bahr, 1992). These studies also found that exercise intensity has a direct effect on EPOC as well (Gaesser and Brooks, 1984; Gore and Withers, 1990a ; 1990b; Bahr, 1992). The effects of varying exercise intensity and duration will be further investigated in Chapter 3.

Numerous authors have reported that EPOC can be separated into rapid and slow components. Krogh and Lindhard (1920) first reported the biphasic nature of EPOC. Hill and associates (1924a; 1924b; 1924c) ascribed the rapid component of EPOC to the oxidative removal of lactate in the muscles and the slow component to the oxidative removal of lactate which had diffused from the muscles to other parts of the body . Margaria *et al.* (1933) modified this idea to include other mechanisms than simply lactate removal. They divided EPOC into a fast "alactacid" component and a slow "lactacid" component. Margaria *et al.* (1933) reported that factors such as phosphagen replenishment, resaturation of myoglobin and hemoglobin molecules with oxygen, and the energetic cost of increased levels of circulation and ventilation could account for the alactacid component. They attributed the reconversion of lactate to glycogen as responsible for the lactacid component (Margaria *et al.*, 1933). They

hypothesized that the alactacid component of recovery oxygen consumption would be complete within the first few minutes after exercise, while the lactacid component would be prolonged for hours. Chapter 3 will examine the different components of EPOC and offer possible explanations for these components.

In the next chapter I will introduce the cost of locomotion as it has been traditionally defined in the literature. Chapter 3 will focus on the influence of exercise duration on EPOC following short bursts of activity. Chapters 4 and 5 will be dedicated to the relationship between lactate and catecholamine concentrations following exercise and recovery oxygen consumption. Chapter 6 will focus on my findings concerning the energetic consequences incurred due to short bursts of activity of this reptile. The last chapter of this thesis will present my final conclusions and areas of future research.

## CHAPTER 2:

### THE ENERGETIC COST OF LOCOMOTION

#### Introduction

All forms of animal locomotion, whether it be walking, running, flying, swimming, or hopping require muscular work. In order for an organism's muscles to perform work, they must have energy. This leads to the question as to how much energy is required for an animal to travel a finite distance? This "cost of transport" or "cost of locomotion" has been traditionally measured in a variety of organisms as the amount of energy (in ml O<sub>2</sub>) required to move 1g of animal 1 km (Taylor *et al.*, 1970, Taylor, 1973; Taylor *et al.*, 1982, John-Alder *et al.*, 1983; John-Alder *et al.*, 1986; Heglund and Taylor, 1988; Baudinette 1991). The cost of transport, (C<sub>t</sub>) has traditionally been measured in terms of long duration (20-45 minutes), sustainable locomotion. For example, Taylor *et al.* (1982) ran eight species of artiodactyls for 15-30 minutes on a treadmill. In an earlier study, Taylor *et al.* (1970) ran six species of mammals (5 rodents, 1 canine) for 30 minutes at variable speeds to achieve steady-state oxygen consumption values. John-Alder and co-workers used 10-30 minute bouts of exercise to determine the cost of transport for the large skink, *Trachydosaurus rugosus* (John-Alder *et al.*, 1986). These traditional measurements are very valuable in that they established methods to identify the energetic cost of steady-state transport for numerous animals. Furthermore, these measurements provided a relationship between mass, speed, and the cost of transport.



### The Relationships between mass, running velocity, and the Cost of Transport.

Taylor and co-workers found an allometric relationship between the cost of transport and mass in mammals (Taylor *et al.*, 1970) and birds and mammals (Taylor *et al.*, 1982). The inverse relationship between mass and the cost of transport is described by the equation:

$$C_t = 0.533M_b^{-0.316}; \quad (1)$$

where  $C_t$  has the units  $\text{ml O}_2\cdot\text{kg}^{-1}\text{m}^{-1}$  and  $M_b$  is in kg (Taylor *et al.*, 1982). In 1986, John-Alder and co-workers found a similar equation for lizards:

$$C_t = 4.22M_b^{-0.282}; \quad (2)$$

where  $C_t$  has the units  $\text{ml O}_2\cdot\text{g}^{-1}\text{km}^{-1}$  and  $M_b$  is in g (John-Alder *et al.*, 1986). A similar inverse relationship between mass and the cost of transport has been found in crustaceans (Full and Herreid, 1983) and insects (Full *et al.*, 1990). The relationship between mass and the cost of transport led to further studies as to why small organisms have a higher mass-specific cost of transport than larger organisms. Heglund *et al.* (1974) found stride frequency at the trot-gallop transition speed to be inversely related to mass in mammals. They also showed that to maintain the same speed as a larger animal, a smaller animal would have to increase its stride frequency. Furthermore, the mass specific cost per kg per stride at the trot-gallop transition speed is nearly constant,  $5.34 \text{ KJ}\cdot\text{kg}^{-1}\text{stride}^{-1}$ , over a large range in mass (Heglund and Taylor, 1988).

Another important finding from these initial studies of the cost of transport is that  $V_{O_2}$  increases linearly with running velocity until a maximum  $V_{O_2}$  ( $V_{O_2\text{max}}$ ) is reached (Taylor *et al.*, 1970). By combining this finding with equation 1, Taylor *et al.* (1982), predicted the following equation:

$$V_{O_2}/M_b = 0.533M_b^{-0.316}\cdot v + 0.300M_b^{-0.303}; \quad (3)$$

Where  $V_{O_2}/M_b$  has the units  $\text{ml O}_2\cdot\text{kg}^{-1}\text{m}^{-1}$ ,  $M_b$  is in kg, and  $v$  is in  $\text{m}\cdot\text{s}^{-1}$ . This equation is very useful in that it predicts  $V_{O_2}$  based on the mass of an animal and the speed that it is moving. Taylor *et al.* (1982) came to the conclusion that as mass

increases, the mass specific cost of locomotion (in terms of oxygen consumption), decreases. This agrees with A.V. Hill's predictions that the muscles of small animals work and consume energy at higher rates than those of large animals (Hill, 1950). They also found that the cost of transport decreased to an asymptotic value as speed increases (Taylor *et al.*, 1970; Taylor *et al.*, 1982). These authors called the energetic cost of running near an animal's maximum speed the minimum cost of running,  $M_{run}$  (Taylor *et al.*, 1970). This asymptotic value can be found by plotting steady-state  $\dot{V}O_2$  against the corresponding running velocity. The slope of this line is  $M_{run}$ . However, the usefulness of this relationship is limited in that  $M_{run}$  occurs only near an animal's top speed, which may not occur very regularly. In fact, these same authors found that animals prefer to run at an intermediate speed during a gallop, and not their maximum speed (Taylor *et al.*, 1982).

In my experiments, steady-state levels of oxygen consumption were not attained by my specimens during the short burst of sprint activity. Therefore, an alternate method of deriving the net cost of locomotion had to be developed. For my experiments, I determined the value of the immediate net cost of locomotion,  $IC_1$  by measuring the volume of oxygen consumed during the bout of exercise minus the resting volume of oxygen consumed, and dividing this value by the distance traveled. As will be discussed later, the net volume of oxygen consumed during and following exercise was included in another measurement, the cost of non-sustainable locomotion,  $C_{ns}$ .

As discussed above, the work done by Taylor *et al.* (1970), John-Alder *et al.* (1986), and Heglund and Taylor (1988) on the cost of transport has done much to expand the knowledge on the energetics of locomotion. However, these studies are somewhat limited because they are based on steady-state locomotor behavior of a type that few animals undergo. With the exception of some migrating animals, (i.e. caribou or wildebeest), most animals do not run for an extended duration (> 5 minutes). This

is particularly true of most lower vertebrates. These animals often rely on quick bursts of activity to travel from one place to another. Few studies have examined the energetic costs of these quick bursts of activity. One purpose of this study was to determine the energetic consequences of burst locomotion.

### Chapter 3:

## THE EFFECT OF VARIABLE DURATION OF MAXIMAL SPRINT ACTIVITY ON EPOC

### Introduction

Numerous studies have examined the effects of varying exercise intensity on EPOC magnitude and duration. As stated earlier, Krogh and Lindhard, (1920) reported that EPOC could be separated into rapid and slow components. Margaria *et al.* (1933) reported that the rapid component involved the replenishment of cellular phosphagens and resaturation of myoglobin and hemoglobin with oxygen, as well as increased heart rate and ventilation. They attributed the slow component to reconversion of lactate to glycogen (Margaria *et al.*, 1933). Increased levels of catecholamines, increased rates of triglyceride-fatty acid (TG-FA), substrate cycling, and increased body temperature have been hypothesized to contribute to the slow component (Hagberg *et al.*, 1980; Bahr, 1992). Hagberg *et al.* (1980), Bahr (1992), and Gore and Withers (1990a; 1990b) have reported that both exercise intensity and duration can have profound effects on EPOC. The slow component of EPOC was profoundly affected by intensity and duration (Hagberg *et al.*, 1980). However, the effects on the rapid component were not so clear cut. Hagberg *et al.*, (1980) reported that the rapid component of EPOC was relatively unaffected by the exercise duration, but was related to exercise intensity. The majority of these studies focused on long duration exercise and relatively few studies have looked at EPOC following short bursts of activity. The purpose of my

experiments was to measure the effects of varying duration of very short ( $\leq 60$  sec) bursts of maximal sprint activity in the desert iguana, *Dipsosaurus dorsalis*.

### Effects of Exercise Duration and Intensity on EPOC

Bahr (1992) exercised students and cadets from the Norwegian Military Academy at variable exercise intensities and duration. In their duration experiments, the subjects exercised 20, 40, and 76 minutes at a constant percentage ( $70 \pm 1\%$ ) of  $\dot{V}O_{2\max}$  (Bahr, 1992). They found a linear relationship between exercise duration and total EPOC for a 12 hour observation period following exercise. They provided the following equation for predicting the 12 hour EPOC volume following exercise at 70% or more of  $\dot{V}O_{2\max}$ :

$$y = 0.8 + 0.40 \cdot x; (p < 0.0005) \quad (4)$$

where y is EPOC volume (liters,  $O_2$ ) and x is exercise duration (minutes) (Bahr, 1992).

Bahr (1992) also found an exponential increase in EPOC with increasing exercise intensity. This relationship was illustrated by the equation:

$$y = 0.129 \cdot 10^{0.0305 \cdot x}; (p < 0.0005) \quad (5)$$

where y is EPOC volume (liters,  $O_2$ ) and x is exercise intensity (%  $\dot{V}O_{2\max}$ ). Similar relationships for humans were also found by Gore and Withers (1990a; 1990b). They found a linear relationship between exercise duration and EPOC at exercise intensities above 50% of  $\dot{V}O_{2\max}$  (Gore and Withers, 1990a; 1990b). However, at an exercise intensity of 30% of  $\dot{V}O_{2\max}$ , they found no relationship between EPOC volume and exercise duration (Gore and Withers, 1990a; 1990b). They reported that EPOC increased with increasing intensity. Gore and Withers (1990a; 1990b) also found that exercise intensity accounted for 45.5% of the variance of EPOC. Duration and the interaction between duration and intensity accounted for only 8.9 and 7.7% of the variation respectively (Gore and Withers, 1990a). It appears that the level of muscle activity is more important than the length of the activity for long bouts of exercise.

Perhaps greater amounts of work, that require more of the muscle to be used have physiological consequences different from those associated with lighter work loads. Heavier work loads could elevate catecholamines to more than lighter work loads via a stress response action. A heavier work load could cause more anaerobic metabolism which would elevate lactate levels more than lighter work loads. The increased concentrations of lactate and the catecholamines may lead to increase substrate cycling or increases in other physiological processes (glycogenolysis, gluconeogenesis) which have been proposed as a possible effectors of EPOC (Bahr, 1992). These authors also found no significant relationship between oxygen deficit and EPOC, and in all cases, EPOC was larger than oxygen deficit (Gore and Withers, 1990b).

Hagberg *et al* (1980) found that the rapid component of EPOC was related to exercise workload (50, 65, and 80%  $\dot{V}O_{2max}$ ), but was not related to exercise duration (5 and 20 minutes) at the same intensities. The slow component of EPOC was not significantly effected by duration of exercise at 50 and 65% of  $\dot{V}O_{2max}$ , but was effected by exercise duration at an intensity of 80%  $\dot{V}O_{2max}$  (Hagberg *et al.*, 1980). From these studies it appears clear that for prolonged exercise at intensities above 50%  $\dot{V}O_{2max}$ , there appears to be a linear relationship between EPOC and exercise duration in humans.

Few studies have investigated the consequences of brief exercise. Zanconato *et al.* (1991) used 1 minute of variable intensity exercise to study EPOC in human adults and children. These authors reported that  $\dot{V}O_2$  recovery time was significantly longer in children following the highest work rate (125%  $\dot{V}O_{2max}$ ) when compared to the lower work rates (50-100%  $\dot{V}O_{2max}$ ) (Zanconato *et al.*, 1991). In adults, these authors found that recovery time increased between the two lowest work rates (50 and 80%  $\dot{V}O_{2max}$ ), but was independent of work rate at the higher intensity exercise (100-125%  $\dot{V}O_{2max}$ ). Thus, the relationship between exercise duration and intensity is unclear for short duration exercise. In adults, lower work rates had effects on EPOC, while EPOC was

independent of intensity at the higher work rates. However, in children, the opposite was reported in that EPOC was independent of lower intensity exercise, but increased significantly after 1 minute of work at 125%  $\dot{V}O_{2\max}$  (Zanconato *et al.*, 1991). EPOC does not appear to be a phenomenon confined only to humans following exercise.

Powers *et al.* (1987) reported the effects of submaximal exercise on oxygen deficit and oxygen debt in ponies. These authors ran their animals for 8 minutes at 2 different work rates (50m/min at 6% grade and 70m/min at 12% grade). Using the  $\dot{V}O_{2\max}$  data from *et al.* (1981), they assumed their experimental work loads to represent 17 and 27% of  $\dot{V}O_{2\max}$ , respectively. Powers *et al.* (1987) reported that  $O_2$  deficit was significantly lower than  $O_2$  debt for both exercise intensities. They also reported that the EPOC following the moderate work rate was significantly larger (76%) than the EPOC of the low work rate (Powers *et al.*, 1987). The relationship between EPOC and exercise intensity and duration was further investigated in canine muscle (Welch *et al.*, 1967). These authors stimulated the gastrocnemius-plantaris muscle group at twitch rates of 0.5-10 twitches/sec for 5, 10, 20, or 60 minutes. They found that oxygen debt increased with increasing twitch rate to a maximum value found at about 5 twitches/sec, but decreased at successively higher twitch rates. This may be attributed to the fact that at higher twitch rates, (5 twitches/second), the muscles were not able to achieve and maintain peak tension (Welch *et al.*, 1967). These authors also reported that EPOC was independent of contraction period. The results of the experiments of Welch *et al.* (1967) and Powers *et al.* (1987) illustrate that the relationship between exercise intensity and duration is not as straightforward in these animals as describe for humans. Additionally, these experiments have concentrated on long term (>5 minutes) bouts of exercise. The purpose of my first experiment was to investigate the relationship between variable duration of short sprint activity and EPOC in an ectotherm, *D. dorsalis*.

## Materials and Methods

### Animal care

Desert iguanas, *Dipsosaurus dorsalis* collected near Palm Springs, CA (July-August 1995) with permission from the California Department of Fish and Wildlife, were transported to Colorado and separated by gender. Eleven, non-reproductive females were selected (mass 26.0-36.8g, mean =  $30.7 \pm 2.52$ g) and placed in cages with a 24 hour photothermal gradient. Animals were fed twice weekly on a diet of chopped lettuce and rat chow. Water was provided *ad libitum*. This population of females was used for all experiments in the following chapters. The experimental protocols used in this thesis were approved by the animal care committee.

### Exercise Protocol

Exercise was conducted on a motorized, thermally equipped treadmill with variable speed control (0-3.6 km/h). One and a half hours before each exercise bout, animals were fitted with a mask as described below and placed on the treadmill surface. An opaque plastic container was placed over the lizards to prevent them from being disturbed. Animals were induced to sprint on the treadmill by gently prodding the hindlimbs and tail. Treadmill speed was adjusted by the experimenter to match the speed of the lizard and to maximize sprint performance. Each animal sprinted for 5, 15, 30, and 60 seconds. Sequence of sprint duration was determined randomly. Upon termination of each sprint, each animal was allowed to recovery quietly on the treadmill for three hours. Expired gases were collected before, during, and after the sprint and metabolic rate determined as mentioned below. No animal was exercised more than once every two days.



### Measurement of Resting Metabolic Rate

For metabolic measurements of resting animals, the animals were fitted with masks as described below and kept in a temperature controlled box for four hours on three different days. Expired gases were collected and  $V_{O_2}$  calculated using the methods mentioned below. The lowest 15 minute average from each of the three resting periods were averaged together to obtain a resting metabolic rate of oxygen consumption for each animal.

### Expired gas collection and measurement of $V_{O_2}$ and $V_{CO_2}$ .

Before each metabolic rate experiment, the lizards were placed in a temperature controlled box at  $40 (\pm 1.0^\circ\text{C})$  (their preferred body temperature, DeWitt (1967)) 4-5 hours before conducting a sprint. One and a half hours before the beginning of exercise, the animal was fitted with a lightweight, transparent, loose-fitting plastic mask attached at the neck. The lizard was then placed on the treadmill surface and covered by a plastic container where it was left undisturbed until the sprint took place or for the entire rest time period. The treadmill temperature was regulated at  $40 (\pm 1.0^\circ\text{C})$ .

Oxygen and carbon dioxide gas concentrations were measured via an Applied Electrochemistry S-3/A Oxygen analyzer and an Anarad Carbon dioxide analyzer, respectively. Gas analyzers were calibrated with 1.015%  $\text{CO}_2$  (balanced  $\text{N}_2$ ) and  $\text{CO}_2$  and  $\text{H}_2\text{O}$  free outside air (20.95%  $\text{O}_2$ ) before the beginning and at intervals during each measurement period. Expired gases were collected using a mask. Air was drawn into the mask and across the animal and then into Tygon tubing. An open flow respirometry system was used to measure metabolic rates of the specimens. Gas flow was regulated by a Tylan RO-32 mass flow meter (using flow rates of 180-650 ml/min). Expired air was passed through Drierite to remove water vapor before entering the gas analyzers. Data acquisition from the flow controller, gas analyzers, and treadmill was accomplished by a National Instruments Lab-NB data acquisition

board connected to an Apple Power Macintosh computer. Using LabVIEW 3.1.1, a graphical programming language, programs were written to record the outputs of the gas analyzers, treadmill, and flow meter. These programs also compute and record instantaneous  $\dot{V}O_2$  and  $\dot{V}CO_2$  using the equations of Withers (1973) and Bartholomew *et al.* (1981). The response time of this system was 7 seconds from the mask to the gas analyzers at a flow rate of 650 ml/minute. This delay was accounted for in measurements of  $\dot{V}O_2$  during exercise and recovery.

### Calculation of EPOC

EPOC was determined by comparing the post-exercise metabolic rate for the lizards to the resting rate of that animal. The end point of recovery was defined as occurring when the 5 minute average post-exercise  $\dot{V}O_2$  equaled or fell below the sample mean resting value plus two standard deviations of the sample mean (mean + 2-SD) (Fig. 1). In other words, I interpreted the endpoint of recovery as occurring when recovery  $\dot{V}O_2$  fell within the 95% upper confidence interval of  $\dot{V}O_{2rest}$  for these animals. To determine the volume of oxygen consumed after the sprint or net EPOC volume (volume B, Fig. 1), the average  $\dot{V}O_2$  over the recovery time period was multiplied by the recovery time as follows:

$$\text{Net EPOC volume} = (\dot{V}O_{2EPOC} - \dot{V}O_{2rest}) * t_{EPOC}; \quad (6)$$

Where Net EPOC volume has the units of ml  $O_2/g$ ,  $\dot{V}O_{2EPOC}$  and  $\dot{V}O_{2rest}$  have the units of ml  $O_2/(g*h)$ , and  $t_{EPOC}$  is in hours. The oxygen consumed during exercise or net exercise volume, (volume A, Fig. 1) was calculated in an analogous manner:

$$\text{Net Exercise volume} = (\dot{V}O_{2exer} - \dot{V}O_{2rest}) * t_{exer}; \quad (7)$$

Where Net exercise volume has the units of ml  $O_2/g$ ,  $\dot{V}O_{2exer}$  and  $\dot{V}O_{2rest}$  have the units of ml  $O_2/(g*h)$ , and  $t_{exer}$  is in hours. The total amount of oxygen consumed above resting values during and after exercise, (net total volume) can be found via the

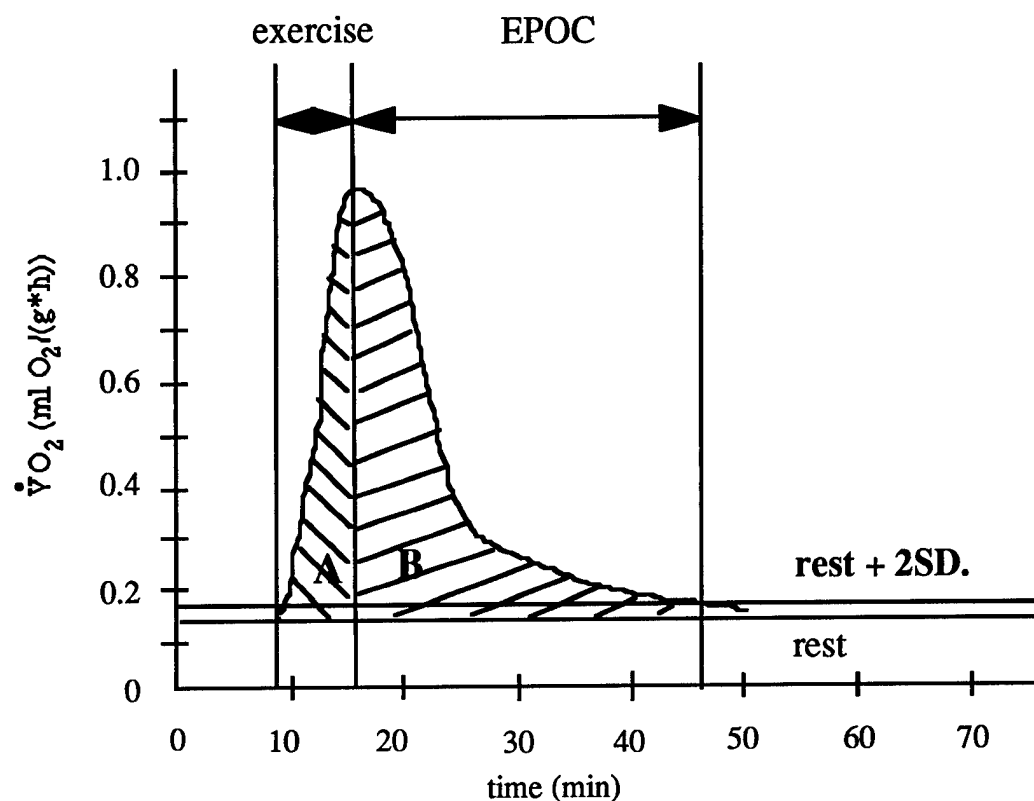


Figure 1. A hypothetical response to exercise used to illustrate the experimental variables measured in this thesis. In order to illustrate exercise volume, exercise begins at 9 minutes and ends at 16 minutes. Volume A is the net volume of  $\text{O}_2$  consumed during exercise. Volume B is the net volume of  $\text{O}_2$  consumed during recovery. EPOC duration is the length of time from the end of exercise until the time that  $\dot{V}O_2$  returns to rest + 2SD value.

following equation:

$$\text{Net Total volume} = \text{Net Exercise volume} + \text{Net EPOC volume}; \quad (8)$$

where all three volumes have the units of ml O<sub>2</sub>/g.

### EPOC Interval Measurements

To measure the effect of exercise duration on the rapid and slow components of EPOC, I divided EPOC into 6, 10 minute segments (1-10, 11-20, 21-30, 31-40, 41-50, and 51-60 minutes of recovery, Fig. 2). These intervals were sequentially numbered 1-6. The volumes of these intervals were found by taking the average V<sub>O2</sub> of the interval and subtracting resting V<sub>O2</sub>. This value was then multiplied by 10 minutes to obtain the net volume of oxygen for that interval.

### Statistical measurements

Repeated measures analysis of variance (ANOVA) and Student's paired t-tests were used to distinguish differences between the different measured variables with duration length as the independent value. For t-tests, an  $\alpha$  value of 0.008512 was used to keep experimentwise error at 5%, as calculated from the following equation:

$$\alpha = 1 - (1 - EE)^{1/k} \quad (9)$$

where EE is experimentwise error in decimal form and k is the number of comparisons made within each experiment. Linear regression statistics were performed to attempt to quantify the relationship between exercise duration and EPOC, distance covered during the sprint, and other variables. Correlation statistics and Fisher's r to z transformations were used to determine if the small differences in mass of these animals had any effect on EPOC. Statistical analysis was performed using the Statview 4.0 program, (Abacus Inc.) on an Apple Power Macintosh.

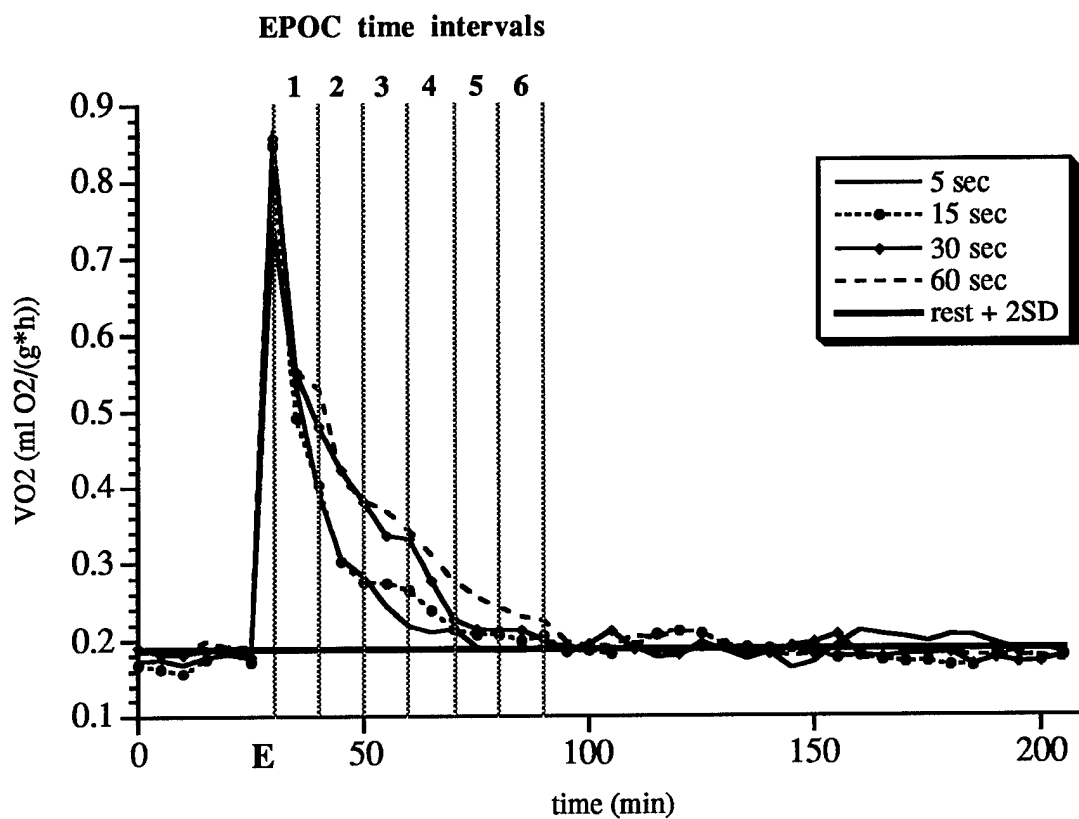


Figure 2. Oxygen consumption of *D. dorsalis* before and after short sprints of 5-60 second duration. Animals began exercise at 30 minutes (E) and were allowed to recover for 180 minutes. Five minute means of 11 animals for each exercise duration are plotted. Note EPOC time intervals 1-6.

## Results

The resting metabolic rate values of each animal are given in table 1. The maximum resting metabolic rate for an animal was 0.1912 ml O<sub>2</sub>/(g\*h) (lizard 1, rest trial 1). However, this was the only resting value that fell outside the upper or lower 95% confidence intervals (mean  $\pm$  2-SD value). The sample mean of these animals was 0.1568 ml O<sub>2</sub>/(g\*h) and the standard deviation was 0.0160 ml O<sub>2</sub>/(g\*h). Therefore, the resting value used to distinguish the end of recovery for these experiments (mean + 2SD), was 0.1888 ml O<sub>2</sub>/(g\*h).

Table 1. Resting metabolic rates of the experimental sample of lizards.

Lizard	Trial			mean	SD	rest + 2SD
	1	2	3			
1	0.1912	0.1651	0.1560	0.1708	0.0183	0.2073
2	0.1730	0.1586	0.1652	0.1656	0.0072	0.1800
3	0.1467	0.1602	0.1558	0.1542	0.0069	0.1681
4	0.1440	0.1403	0.1370	0.1404	0.0035	0.1474
5	0.1493	0.1292	0.1364	0.1383	0.0102	0.1588
6	0.1721	0.1702	0.1737	0.1720	0.0017	0.1755
7	0.1811	0.1623	0.1661	0.1698	0.0099	0.1897
8	0.1350	0.1254	0.1350	0.1318	0.0056	0.1429
9	0.1475	0.1561	0.1738	0.1591	0.0134	0.1860
10	0.1762	0.1622	0.1573	0.1653	0.0098	0.1848
11	0.1548	0.1706	0.1478	0.1577	0.0117	0.1810
sample				<b>0.1568</b>	<b>0.0160</b>	<b>0.1888</b>

Metabolic rate has the units ml O<sub>2</sub>/(g\*h). Numbers in boldface are the values for the entire sample of eleven animals (33 trials).

A typical response to sprint activity is presented in Fig. 3. As the animal began to sprint, V<sub>O2</sub> rapidly increased. Following the termination of exercise, V<sub>O2</sub> slowly returned back to resting levels. Levels of V<sub>O2</sub> during exercise (exercise V<sub>O2</sub>), are given in Table 2. Exercise V<sub>O2</sub>, (V<sub>O2</sub><sub>exer</sub>) increased slightly with sprint duration from a low of 1.31 ml O<sub>2</sub>/(g\*h) for 5 seconds of exercise and a high of 1.49 ml O<sub>2</sub>/(g\*h) for

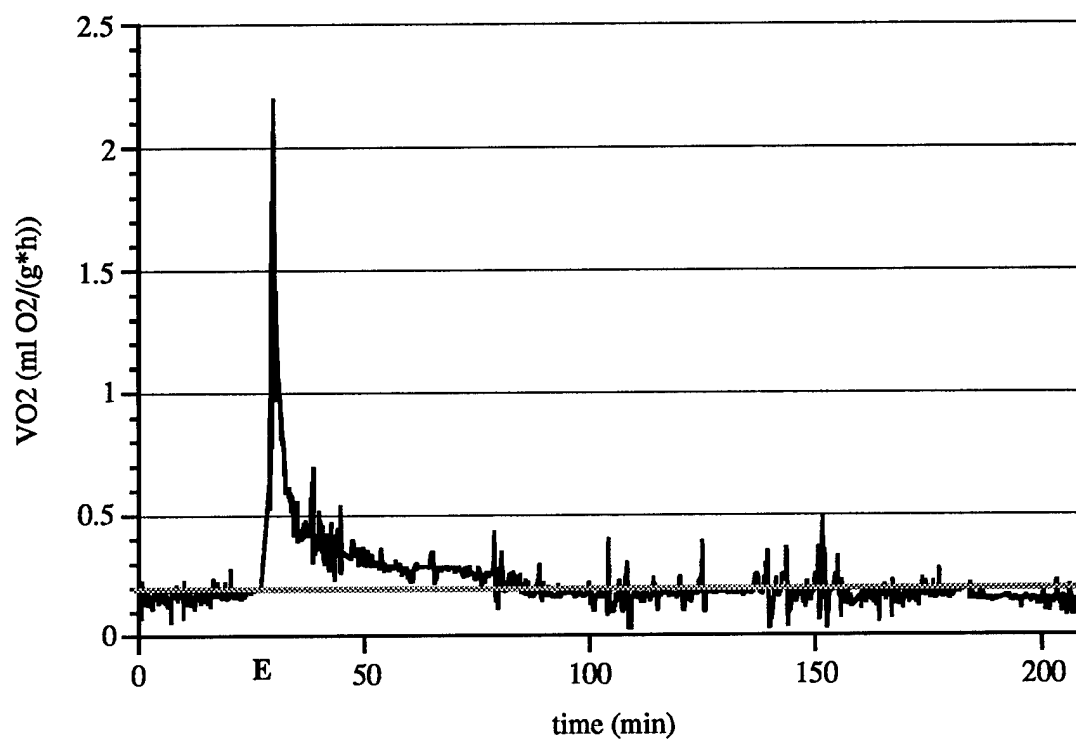


Figure 3. Sample trace of  $\dot{V}O_2$  before, during, and after a typical 60 second sprint.  $\dot{V}O_2$  is in ml  $O_2$ /(g\*h)). The beginning of exercise is represented by E below the x-axis.

Table 2. Summary of metabolic variables and distance traveled for each sprint duration.

Sprint duration (sec)	Exercise $\dot{V}O_2$ (ml $O_2$ /g/h)	EPOC duration (min)	net vol run (ml $O_2$ /g)	net vol EPOC (ml $O_2$ /g)	distance (meters)
5	1.31 $\pm$ 0.12	32.73 $\pm$ 2.10 <b>a</b>	0.0016 $\pm$ 0.0002 <b>a</b>	0.1339 $\pm$ 0.0131 <b>a</b>	3.5 $\pm$ 0.2 <b>a</b>
15	1.41 $\pm$ 0.15	42.95 $\pm$ 5.13 <b>a</b>	0.0052 $\pm$ 0.0006 <b>b</b>	0.1559 $\pm$ 0.0205 <b>a</b>	9.2 $\pm$ 0.7 <b>b</b>
30	1.43 $\pm$ 0.09	52.08 $\pm$ 3.91 <b>b</b>	0.0106 $\pm$ 0.0007 <b>c</b>	0.2181 $\pm$ 0.018 <b>b</b>	15.5 $\pm$ 0.8 <b>c</b>
60	1.49 $\pm$ 0.09	62.68 $\pm$ 2.48 <b>c</b>	0.0222 $\pm$ 0.0015 <b>d</b>	0.2475 $\pm$ 0.0183 <b>b</b>	27.3 $\pm$ 2.0 <b>d</b>
	p=0.7528	p<0.0001	p<0.0001	p<0.0001	p<0.0001

Values are mean  $\pm$ SEM (n=11). Different superscript letters within columns are significantly different.



60 seconds of sprinting. However, there were no statistical differences in the exercise  $\dot{V}O_2$  values ( $p = 0.7528$ ). Although, there were not any significant differences in the mean  $\dot{V}O_2$  during the sprint, there were differences in the net volume of oxygen consumed during the sprint (net vol. run) (Table 2). Animals consumed almost 14x as much oxygen during a 60 second sprint, than they did during a 5 second sprint (0.0222 and 0.0016 ml  $O_2/g$ , respectively). Following sprint duration of 5, 15, 30, and 60 seconds, *D. dorsalis* exhibited a prolonged EPOC (Table 2, Fig. 2).

The duration of sprint activity had a significant effect on the duration of EPOC. Following a 5 second sprint, recovery lasted for  $32.7 \pm 2.10$  min., while recovery from a 60 second dash required  $62.7 \pm 2.48$  min. (Table 2). Exercise duration had a positive effect on EPOC duration. The equation for the best fit, least squares linear regression of this data is:

$$y = 33.4 + 0.52x; r^2 = 0.4578 \quad (10)$$

where y is EPOC duration (min) and x is sprint duration (sec) (Fig. 4). Although the data were highly variable, the general trend of increasing EPOC duration with increasing sprint duration can be seen. The differences reported in EPOC length were also manifested in the volume of oxygen consumed during recovery.

Sprint duration had a significant effect on the net volume of oxygen consumed during recovery (net EPOC volume, Table 2, Fig. 5). Once again, as exercise duration increased, net EPOC volume increased as well. Following a 5 second sprint, the animals consumed an average of 0.134 ml  $O_2/g$  body weight above the resting level of oxygen consumption during recovery. The net volume of oxygen consumed during recovery increased to 0.248 ml  $O_2/g$  following a 60 second sprint. As seen in the EPOC duration comparison to exercise duration, there existed a positive relationship between exercise duration and the net volume of EPOC. The best fit linear equation for these data is:

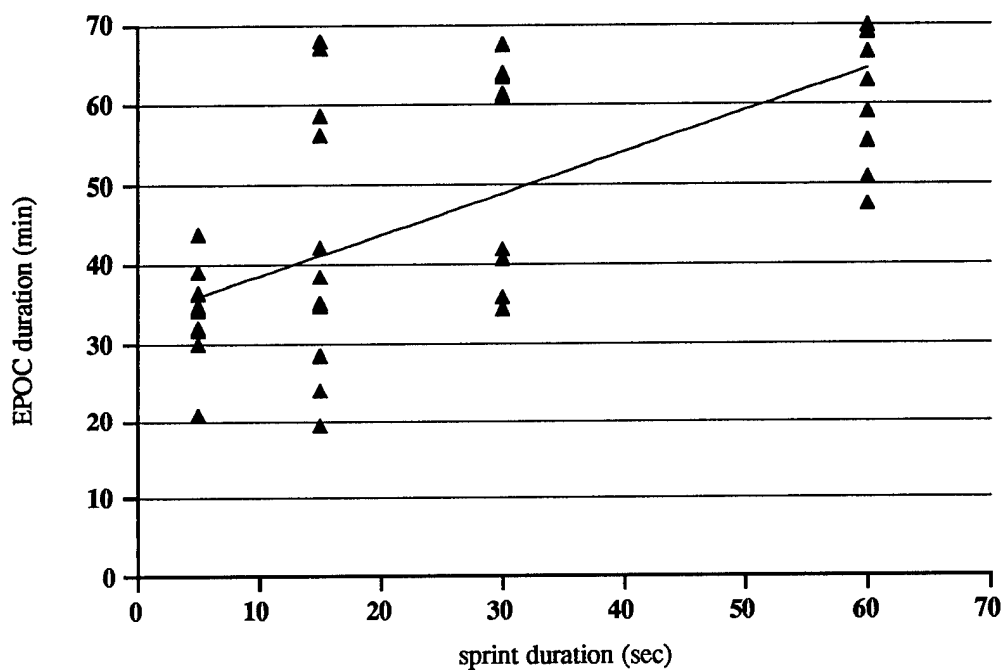


Figure 4. The relationship between sprint duration and EPOC duration. Line represents the best fit linear regression:  $\text{EPOC (min)} = 33.4 + 0.52 \cdot \text{exercise duration (sec)}$ ;  $r^2 = 0.4578$ . ANOVA ( $P < 0.0001$ ). Individual points are plotted for all 11 animals.

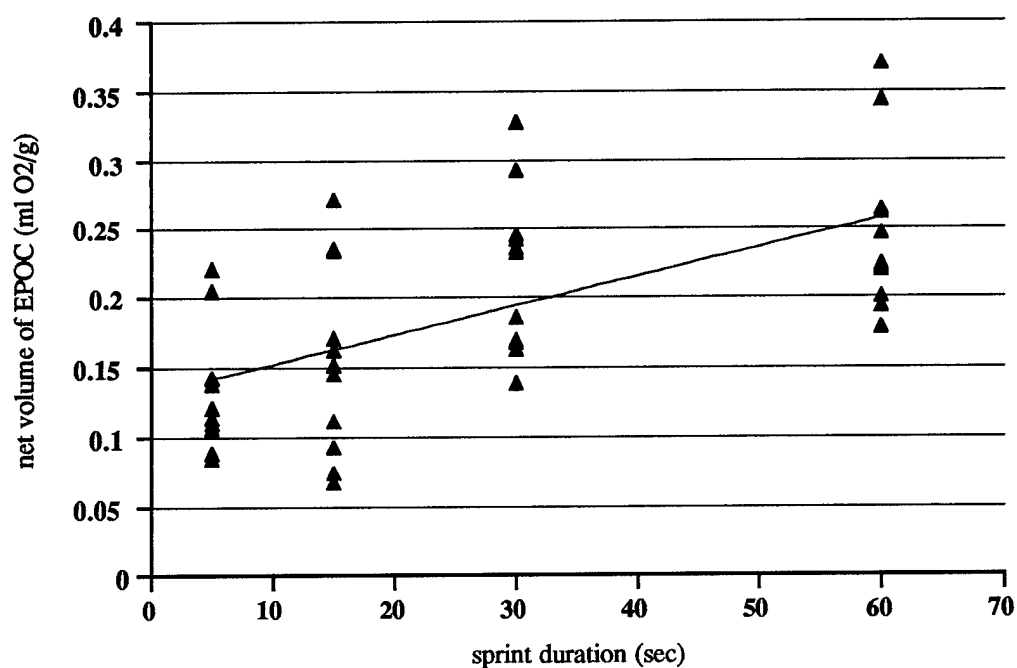


Figure 5. The relationship between sprint duration and net EPOC volume. Line represents the best fit linear regression: net EPOC volume (ml O<sub>2</sub>/g) = 0.131 + 0.0021-exercise duration (sec);  $r^2 = 0.3661$ . ANOVA ( $P < 0.0001$ ). Individual points are plotted for all 11 animals.

$$y = 0.131 + 0.0021 \cdot x; r^2 = 0.3661$$

(11)

where y is net volume of EPOC (ml O<sub>2</sub>/g) and x is sprint duration (sec).

Mass had no effect on EPOC duration or the net volume of EPOC (Table 3).

Table 3. Correlation table between mass and the net volume of EPOC and EPOC duration.

	mass (correlation value)	mass (p value)
EPOC vol 5 sec	-0.399	0.2315
EPOC vol 15 sec	0.110	0.7543
EPOC vol 30 sec	0.397	0.2351
EPOC vol 60 sec	0.420	0.2050
EPOC dur 5 sec	-0.256	0.4591
EPOC dur 15 sec	0.314	0.3588
EPOC dur 30 sec	0.197	0.5722
EPOC dur 60 sec	0.232	0.5048

Correlation values are from a correlation comparison (0 = no correlation, 1 = perfect direct linear relationship, -1 = perfect indirect linear relationship. P-values are the Fisher's r-z transformation.

There was a linear relationship between distance traveled and sprint duration (Table 2, Fig. 6). The average distances traveled during a 5, 15, 30, or 60 second sprint were 3.5, 9.2, 15.5, and 27.3 meters, respectively. The animals traveled 3x as far during the 15 second sprint and 7.8x as far during the 60 second sprint compared to the 5 seconds sprint. The average speeds achieved during these sprints were 0.7, 0.61, 0.52, and 0.46 m/s for the 5, 15, 30, and 60 sprints respectively. The slope of the linear relationship between sprint duration and distance traveled represents the average speed (0.425), attained by the animals during all of the sprints. The fact that the average speed attained by these lizards is less than the maximum speed achieved during the 5 second sprint, suggests that the animals cannot maintain a constant sprint speed for an extended time period. Most of the animals slowed down after about 25-30 seconds of sprinting. The animals would still run on the treadmill, but they would not

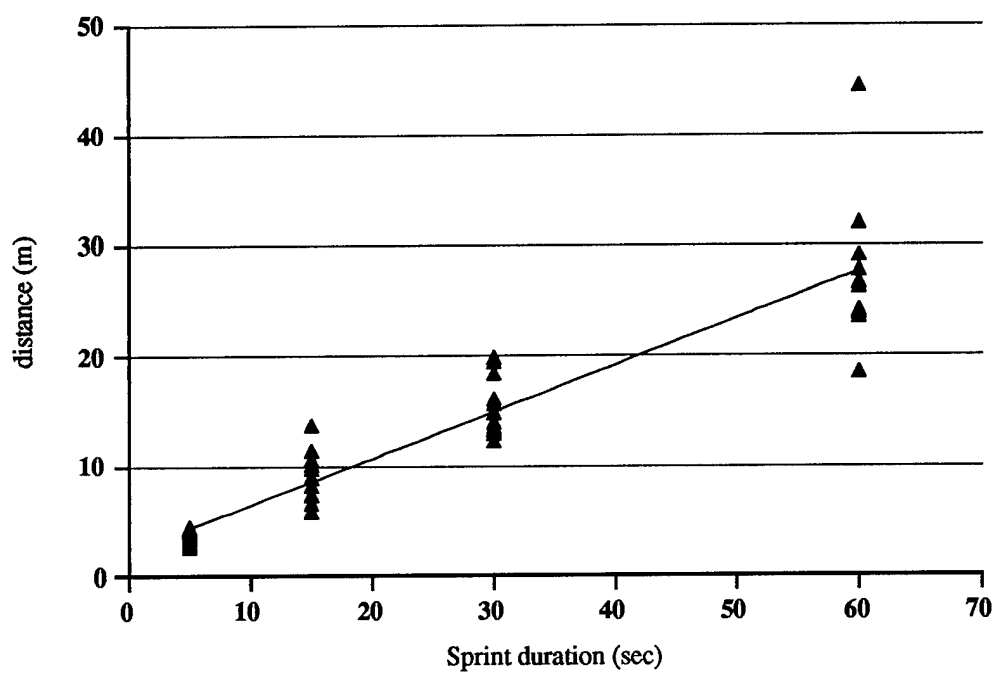


Figure 6. The relationship between sprint duration and distance traveled. Line represents the best fit linear regression: distance (m) = 2.19 + 0.425-exercise duration (sec); ( $r^2 = 0.8533$ ). ANOVA ( $P < 0.0001$ ). Individual points are plotted for all 11 animals.

achieve the bipedal running style seen at the beginning of the sprints. However, the positive relationship between sprint duration and distance traveled illustrates that the animals are performing more work during the longer sprints. Therefore, the longer the animal sprints the greater the distance will be that the animal covers.

To investigate the possibility of any differences in the time course of recovery following sprints of different duration, I separated EPOC into six, consecutive, ten-minute time intervals labeled 1-6 as in Fig. 2. Interval 1 is the first 10 minutes of EPOC (minutes 1-10), interval 2 is the second 10 minutes of EPOC (11-20) and so on, where the final interval, #6, is the sixth 10 minutes of EPOC (51-60). For the purposes of this thesis, interval 1 will be considered the rapid interval of EPOC and intervals 2-6 will be considered the slow intervals of EPOC. I compared the net volume of oxygen consumed during these intervals and summarized the results in table 4.

Table 4. Volume of oxygen consumed during the different intervals of EPOC for the 4 different exercise regimens.

Exercise Duration	Interval		
	1	2	3
5	0.087±0.006	0.035±0.005 <b>a</b>	0.020±0.005 <b>a</b>
15	0.096±0.008	0.037±0.004 <b>a</b>	0.020±0.004 <b>a</b>
30	0.097±0.007	0.052±0.003 <b>b</b>	0.037±0.006 <b>a,b</b>
60	0.100±0.006	0.055±0.004 <b>b</b>	0.039±0.004 <b>b</b>
Exercise Duration	Interval		
	4	5	6
5	0.010±0.001 <b>a</b>	0.009±0.002 <b>a</b>	0.004±0.002 <b>a</b>
15	0.016±0.003 <b>a</b>	0.010±0.003 <b>a,b</b>	0.008±0.002 <b>a,b</b>
30	0.027±0.006 <b>a,b</b>	0.012±0.003 <b>a,b</b>	0.010±0.003 <b>a,b</b>
60	0.030±0.003 <b>b</b>	0.020±0.002 <b>b</b>	0.014±0.002 <b>b</b>

Values are mean ± 1 SEM (n=11) volume of oxygen consumed in ml O<sub>2</sub>/g. Values within the same column with different superscript **letters** are significantly different (students t-test, P<0.008512).

There were no statistical differences seen in the first 10 minute interval, (interval 1) for any exercise duration. However, there were differences in EPOC intervals 2-6 (Table 4). Following a 5 second sprint, the animals consumed a net volume of oxygen of 35  $\mu\text{l O}_2$  per gram of body weight during interval 2. This value increased significantly ( $P=0.0051$ ), to 55  $\mu\text{l O}_2$  per gram following a 60 second sprint. The 5 second volume for this interval was also lower than the 30 second exercise volume (52  $\mu\text{l O}_2$  per gram). The net volume consumed during interval 2 following 15 seconds of exercise was significantly lower than after 30 and 60 second sprints ( $P=0.0055$  and 0.0004, respectively). The trend of consuming an greater amount of oxygen following longer exercise bouts persisted in the other late intervals. For intervals 3 and 4, a statistically smaller amount of oxygen was consumed after 5 and 15 second sprints compared to the volume of oxygen consumed following 60 seconds of exercise. For intervals 5 and 6, only the volume of oxygen consumed following the 5 second sprints was significantly lower than the net volume following 60 second sprints (Table 4).

A majority of the oxygen consumed by *D. dorsalis* following these short bursts of activity was consumed during the first 10 minutes of recovery, interval 1 (Table 5).

Table 5. Net volume of oxygen consumed during the early and late intervals of EPOC for the 4 different exercise regimens.

sprint duration	interval	
	1	2-6
5	86.9	78.4
15	96.1	91.8
30	97.5	137.3
60	99.7	158.1

Values are mean ( $n=11$ ) volume of oxygen consumed in  $\mu\text{l O}_2/\text{g}$ .

Following 5 and 15 second sprints, these animals consumed more net oxygen during the first 10 minutes of recovery that they did during the latter 50 minutes of recovery.

Following 30 and 60 second sprints, these animals consumed more net oxygen during intervals 2-6 of recovery, than interval 1, but the net volume of oxygen consumed during interval 1 represented a large percentage of the net total volume of EPOC (42 and 39%, respectively).

### Discussion

Oxygen consumption was elevated above resting levels for 33 minutes following only 5 seconds of sprint activity in *D. dorsalis*. This is a remarkable response to such a short bout of exercise. Recovery was prolonged even more following longer sprints and represents a positive relationship between sprint duration and EPOC duration (Fig. 4). EPOC duration increased significantly following the 30 and 60 second sprints (52 and 63 minutes, respectively) compared to the 5 and 15 second sprints (33 and 43 minutes, respectively, Table 2). It appears that for this type of activity, the very act of running a short distance can cause a prolonged EPOC. Additionally, the longer these animals sprint, the longer time that oxygen consumption remains elevated above resting levels. This finding agrees with the results of other authors who found that exercise duration had a direct effect on recovery time at exercise of intensities above 50% of  $V_{O2max}$  (Hagberg *et al.*, 1980; Gore and Withers, 1990a; 1990b; Bahr, 1992) and above 125% of  $V_{O2max}$  (Zanconato *et al.*, 1991). In my experiments, *D. dorsalis* are sprinting at speed above their MAS and therefore, the positive relationship between exercise duration and EPOC is expected.

Wagner and Gleeson (1996) found  $V_{O2}$  to remain elevated above resting levels in *D. dorsalis* for 90-120 minutes after 5 minutes of exhaustive treadmill exercise. By using equation 10, 5 minutes of sprint activity would predict an EPOC length of 189 minutes. This value is substantially higher than that reported by Wagner and Gleeson (1996). The reason for this discrepancy may be that *D. dorsalis* cannot sprint maximally for 5 minutes. As stated earlier, these animals tire quickly on the treadmill



and their performance begins to degrade after approximately 30-45 seconds of sprint activity. Another explanation of this discrepancy is that the average speed of *D. dorsalis*, (0.37 m/s), in the Wagner and Gleeson (1996) study was lower than the speed achieved during the 60 second sprints (0.46 m/s) and a little more than half of the speed during the 5 second sprints (0.70 m/s). Therefore, the animals in the Wagner and Gleeson (1996) were not able to sprint for the full 5 minutes and this may have reduced the recovery time from the predicted value. Another reason for the discrepancy is that equation 10 is used to predict EPOC after brief bouts of exercise. Equation 10 may not hold for longer sprints, but the relationship between EPOC duration and extended sprint duration is probably similar to that found between EPOC and shorter sprints of exercise. During the prolonged period of EPOC seen in these animals following brief bouts of exercise, a large volume of oxygen was consumed above resting levels.

The net volume of oxygen consumed during the 33 minutes of recovery following 5 seconds of sprint activity, (0.134), is close to 84x greater than the net volume of oxygen consumed during exercise (0.0016 ml O<sub>2</sub>/g). The net volume of oxygen consumed during recovery represents a large portion of the net total volume of oxygen consumed during exercise and recovery (net total volume; net exercise volume + net EPOC volume). Following a 5 second sprint, these animals consumed 98.7% of the net total volume of O<sub>2</sub> during recovery. The percentage of the net total volume of oxygen consumed during the sprint and recovery decreased following longer sprints (97.5, 96.6, and 93.2% following 15, 30, and 60 second sprints, respectively). The percentage data presented here can be compared to the findings from earlier studies which found that the volume of EPOC represented only 1-10% of the net volume of oxygen consumed during extended bouts of exercise (Gore and Withers, 1990a ; 1990b; Bahr, 1992). Using a one minute exercise regimen, Zanconato *et al.* (1991) found that the volume of EPOC represented >50% of the net volume of oxygen

consumed during and after exercise. Therefore, it may be concluded that EPOC represents a substantial energetic cost to *D. dorsalis*, especially for short bursts of activity. Additionally, as the duration of locomotor activity becomes shorter, the more significant the net volume of EPOC becomes to the total amount of oxygen consumption.

The energetic consequences of a prolonged EPOC represent a true cost to the animal and are ecologically relevant. Some animals in the field often sprint for short distances at speeds near their maximum aerobic speed (Kenagy and Hoyt, 1989). As a consequence of these short bursts of sprint activity, animals probably experience a prolonged EPOC. Because of the short duration of the sprints observed in animals in the wild, the majority of the oxygen consumed by these animals probably occurs after locomotion has ceased. Therefore, the energetic costs of these short sprints may be underestimated if EPOC is not included in the energetic cost. Chapter 6 will focus on the energetic consequences of short term bouts of exercise and the impact EPOC has on these energetic costs.

The net volume of oxygen consumed during recovery in these animals was positively related to sprint duration. These data agree with the findings in humans by other authors (Hagberg *et al.*, 1980; Gore and Withers, 1990a; 1990b; Zanconato *et al.*, 1991; Bahr, 1992). It appears that the greater amount of work done by the muscles after longer sprints has physiological consequences which affect EPOC. The longer sprints may be increasing lactate or catecholamines levels more than shorter sprints, which might in some way prolong EPOC. The potential effects of lactate and catecholamine concentrations on EPOC will be investigated in the next two chapters.

The differences in net EPOC volume after different duration of exercise were not as pronounced as the differences in EPOC duration. However, there was still a significant increase in EPOC volume following longer duration sprints. By examining the interval data from my experiments I can find what component of EPOC is being

altered by increasing duration of exercise. There were no statistical differences in the net amount of oxygen consumed during the rapid period of recovery (interval 1) between exercise duration. This agrees with the data of Hagberg *et al.* (1980), who found no differences in the rapid component of EPOC following exercise of variable duration. The amount of oxygen consumed during the first 10 minutes of recovery in my experiments was larger than that consumed during the latter 50 minutes of recovery (intervals 2-6) for 5 and 15 seconds sprints. The net amount of oxygen consumed during interval 1 was quite large when compared to the volume consumed during intervals 2-6, following 30 and 60 seconds of exercise. The differences between sprint duration and net volume of oxygen consumed during the entire EPOC period may have been masked by the rapid interval of EPOC for two reasons. One, the magnitude during EPOC interval 1 represented a large percentage of the net amount of oxygen consumed during the entire EPOC period. Two, there were no statistically significant differences among sprint duration for interval 1. While exercise duration had no effect on the interval 1 of recovery, varying sprint duration did have effects on intervals 2-6 of EPOC.

The net volume of oxygen consumed during intervals 2-6 was positively affected by exercise duration. This agrees with the results from Hagberg *et al.* (1980) who found that for a work load of 80% of  $\dot{V}O_{2\max}$  the net volume of oxygen consumed during the slow component of EPOC following 80 minutes of exercise was 5x greater than that consumed after 20 minutes of exercise. It seems that increasing exercise duration has positive effects on the slow intervals of EPOC, but not the rapid interval. Therefore, the factors contributing to the slow component of EPOC, such as catecholamine levels, lactate removal, and increased heart rate and ventilation, (Bahr, 1992), may be more important than the factors thought to influence the rapid component of EPOC. Chapter 7 will provided a summary of the possible explanations for the prolonged EPOC following increasing duration exercise.

The metabolic rate data from my experiments are similar to previous data collected for *Dipsosaurus dorsalis*. Bennett and Dawson (1972) measured standard metabolic rate at temperatures between 25 and 45°C. They reported a standard  $V_{O_2}$  of approximately 0.15 ml  $O_2/(g \cdot h)$  at 40°C (Bennett and Dawson, 1972). Wagner and Gleeson (1996) found a resting  $V_{O_2}$  of 0.118 ml  $O_2/(g \cdot h)$  for animals at 40°C. These data agree nicely with resting  $V_{O_2}$  found in my animals of 0.157 ml  $O_2/(g \cdot h)$  at 40°C. The small discrepancy between my data and the data of Wagner and Gleeson (1996) may be due to the weight differences between their specimens (mean weight, 57.2g) and mine (mean weight, 30.7g). Further explanation may be found in that Wagner and Gleeson (1996) fasted their lizards for 5-7 days before experiments.

The mean  $V_{O_2}$  during the sprint (exercise  $V_{O_2}$ ) of my animals (1.41 ml  $O_2/(g \cdot h)$ ) was somewhat lower than that reported by Bennett and Dawson (1972) (2.27 ml  $O_2/(g \cdot h)$ ). This may be attributed to the fact that Bennett and Dawson (1972) electrically stimulated their specimens for 120 seconds, while my animals underwent  $\leq 60$  seconds of activity. If my animals would have been allowed to run for longer periods of time, they would likely have achieved a values similar to Bennett and Dawson (1972).

John-Alder and Bennett (1981) derived an equation that predicts  $V_{O_2}$  based on the speed that *D. dorsalis* is locomoting. The exercise  $V_{O_2}$  of my lizards during 5 and 15 second sprints was slightly lower than the predicted values for the speeds of these sprints. This is explained by the fact that my animals were not able to increase  $V_{O_2}$  to steady-state levels before the termination of the sprint. Therefore, the actual  $V_{O_2}$  achieved during these sprints was lower than the  $V_{O_2}$  that would have been achieved under steady-state conditions. Also, the equation of John-Alder and Bennett (1981) was used to best describe submaximal  $V_{O_2}$  and was derived by animals that were performing submaximal exercise, where my animals were performing maximally.

As stated earlier, the speeds attained by my animals are above the maximal aerobic speed (MAS) predicted by John-Alder and Bennett (1981). They predicted an MAS of 0.95 km/h for *D. dorsalis* walking on a treadmill at 40°C. The lowest average speed obtained by my lizards (1.64 km/h) was obtained during the 60 second sprints. This speed is greater than the MAS and indicates that my specimen were sprinting at non-aerobically sustainable speeds. John-Alder and Bennett (1981) predicted that *D. dorsalis* would only be able to sustain the treadmill speeds achieved during my sprinting experiments for less than 2 minutes. My experiments agree with their prediction. During the sprints in my experiments, the animals often achieved bipedal locomotion, but were unable to sustain this type of locomotion for extended periods (>30-45 seconds).

## CHAPTER 4:

### THE EFFECTS OF LACTATE CONCENTRATION ON EPOC

#### Introduction

Lactate accumulations in the muscles following exercise was originally thought to be a major component of the oxygen debt hypothesis (Hill and Lupton, 1923). However, more contemporary studies have questioned this relationship. To test the original lactate oxygen debt hypothesis, Abramson *et al.* (1927) infused non-exercising dogs with sodium lactate. They reported a 23% increase in  $V_{O_2}$  following the infusions, but this did not differ significantly from the increase in  $V_{O_2}$  seen following the control injection of  $NaHCO_3$  alone (Abramson *et al.*, 1927). Alpert and Root (1954) found no correlation between  $V_{O_2}$  and the amount of lactate infused in dogs which supported the findings of Abramson *et al.* (1927). Kayne and Alpert (1964) also found no correlation between recovery oxygen consumption and lactate removal in anesthetized dogs following exercise. More recently, Gaesser and Brooks (1980) reported a temporal dissociation between lactate removal and the slow phase of EPOC in rats. These authors found that lactate returned to resting levels within 15 minutes of the end of exercise, while  $V_{O_2}$  remained elevated for 120 minutes (Gaesser and Brooks, 1980). Harris *et al.* (1968) found a somewhat different situation in humans, in that blood lactate peaked after the cessation of exercise and remained elevated after  $V_{O_2}$  had returned to resting levels. Although this is different of what was reported in rats by Gaesser and Brooks (1980), it still illustrates a temporal disunion between lactate removal and recovery  $V_{O_2}$ .

Numerous studies of ectothermic vertebrates have also found that the removal of lactate and excess post-exercise oxygen consumption are not tightly coupled (Bennett and Licht, 1973; Gratz and Hutchinson, 1977; Hutchinson *et al.*, 1977; Bennett, 1978; Gleeson, 1980; Milligan and McDonald, 1988; Withers *et al.*, 1988b; Wagner and Gleeson, 1996). For example, Wagner and Gleeson (1996) found that in *D. dorsalis*, EPOC ended approximately 90-120 minutes after 5 minutes of exhaustive treadmill exercise, but these lizards had not removed a statistically significant portion of the lactate after 120 minutes of recovery.

Bennett and Licht (1973) measured post-exercise  $V_{O_2}$  and rates of lactate removal in three species of amphibians. They found that in *Hyla regilla* and *Batrachoseps attenuatus*, oxygen consumption had returned to resting levels within 1 hour, but lactate content was still elevated. Withers *et al.* (1988b) found that  $V_{O_2}$  returned to resting levels within 60 minutes in another amphibian, *Bufo americanus*, following 10 minutes of activity. However, whole body lactate concentrations remained elevated 4 hours after exercise (Withers *et al.*, 1988b). Milligan and McDonald (1988) reported that blood lactate levels remained elevated in the starry flounder, *Platichthys stellatus*, after  $M_{O_2}$  had returned to resting levels following exhaustive exercise. These authors found a less straightforward relationship in the coho salmon, *Oncorhynchus kisutch*, however  $M_{O_2}$  did decline despite almost constant blood lactate levels.

The disjunction between excess post-exercise oxygen consumption and the removal of lactate appears to be a phenomenon seen across the spectrum of vertebrate classes. Thus it seems that lactate does not play as significant a role in EPOC as previously thought. However, these many of these previous experiments used exhaustive exercise in their protocols. The purpose of this next experiment was to test the relationship between EPOC and blood lactate concentrations in *D. dorsalis* following variable duration of short, non-exhaustive sprint activity. A major focus of

this experiment was to determine if the disunion between lactate removal and recovery oxygen consumption held for extremely short bursts of activity.

## Materials and Methods

### Animal Care

Animal care was the same as described in Chapter 3.

### Exercise Blood Sampling

A sample of animals ( $n=6$ ) was fitted with carotid cannulas and allowed to recover for 48 hours. Animals were placed in a temperature controlled cabinet, ( $40 \pm 1.0^\circ\text{C}$ ) four hours before the sprint occurred. To exercise the animal, the animals were quickly removed to the treadmill. Immediately thereafter, the animals were induced to sprint for 5, 15, or 60 seconds on the treadmill. Each animal underwent each exercise duration in random order with at least 24 hours between sprints. The animals were then removed from the treadmill and placed back into the container in the temperature controlled cabinet. A cannula extension was added and was threaded through an opening in the container to allow access to it without disturbing the animal. Blood samples ( $25\mu\text{l}$ ), were taken after exercise at intervals of 5, 30, and 60 minutes post-exercise. Blood samples were added to 4 volumes of 6% perchloric acid ( $\text{HClO}_4$ ) and stored at  $-70^\circ\text{C}$ . Lactate concentrations were determined in supernatants according to previous literature (Gleeson, 1985).

### Resting Blood Sampling

To obtain resting blood samples, the animals were placed in the  $40^\circ\text{C}$  cabinet and allowed to rest for 4 hours. At the end of this time period, the animal was seized and a blood sample ( $25\mu\text{l}$ ), quickly taken via the orbital sinus with a  $75\mu\text{l}$  capillary



tube. Sampling was accomplished within 30 seconds. Samples that took longer than 30 seconds to acquire or samples where the animal struggled violently were discarded. Blood samples were added to 4 volumes of 6% perchloric acid ( $\text{HClO}_4$ ) and stored at  $-70^\circ\text{C}$ . Lactate concentrations were determined as described above.

## Results

The resting levels of lactate obtained during this experiment were  $2.5 \pm 0.7$  mmol/kg, (Table 6). Lactate levels increased significantly above resting levels for all three sprint duration (Table 6). Lactate levels were positively affected by sprint

Table 6. Recovery blood lactate concentrations following variable duration of sprint activity.

Exercise Duration (sec)	Time of Recovery (min)		
	5	30	60
5	$8.5 \pm 1.2$ <b>a</b>	$7.8 \pm 1.9$ <b>a,b</b>	$7.2 \pm 1.5$ <b>a,b</b>
15	$13.5 \pm 2.0$ <b>b</b>	$10.4 \pm 2.8$ <b>b</b>	$10.0 \pm 1.8$ <b>b,c</b>
60	$22.3 \pm 4.5$ <b>c</b>	$22.9 \pm 3.1$ <b>c</b>	$14.1 \pm 2.4$ <b>c</b>
rest	$2.5 \pm 0.7$ <b>d</b>	$2.5 \pm 0.7$ <b>d</b>	$2.5 \pm 0.7$ <b>d</b>

Values are mean  $\pm$  1 SEM (n=6) blood concentrations of lactate, mmol/kg. Values within the same column with different superscript **letters** are significantly different (students t-test,  $P < 0.008512$ ).

duration (ANOVA  $P < 0.0001$ ). The peak blood lactate concentration was achieved following the 60 second sprints after 30 minutes of recovery ( $22.9 \pm 4.5$  mmol/kg), compared to only  $7.8 \pm 1.2$  mmol/kg of lactate accumulated following a 5 second sprint at the same recovery time. The levels of blood lactate remained elevated even after  $\dot{V}_{O_2}$  had returned to resting levels (Fig. 7). For all three exercise regimens, oxygen consumption had returned to resting levels following 60 minutes of recovery. However, blood lactate concentrations remained statistically higher than rest after 60 minutes of recovery. After the 5 and 15 second exercise bouts, lactate was not

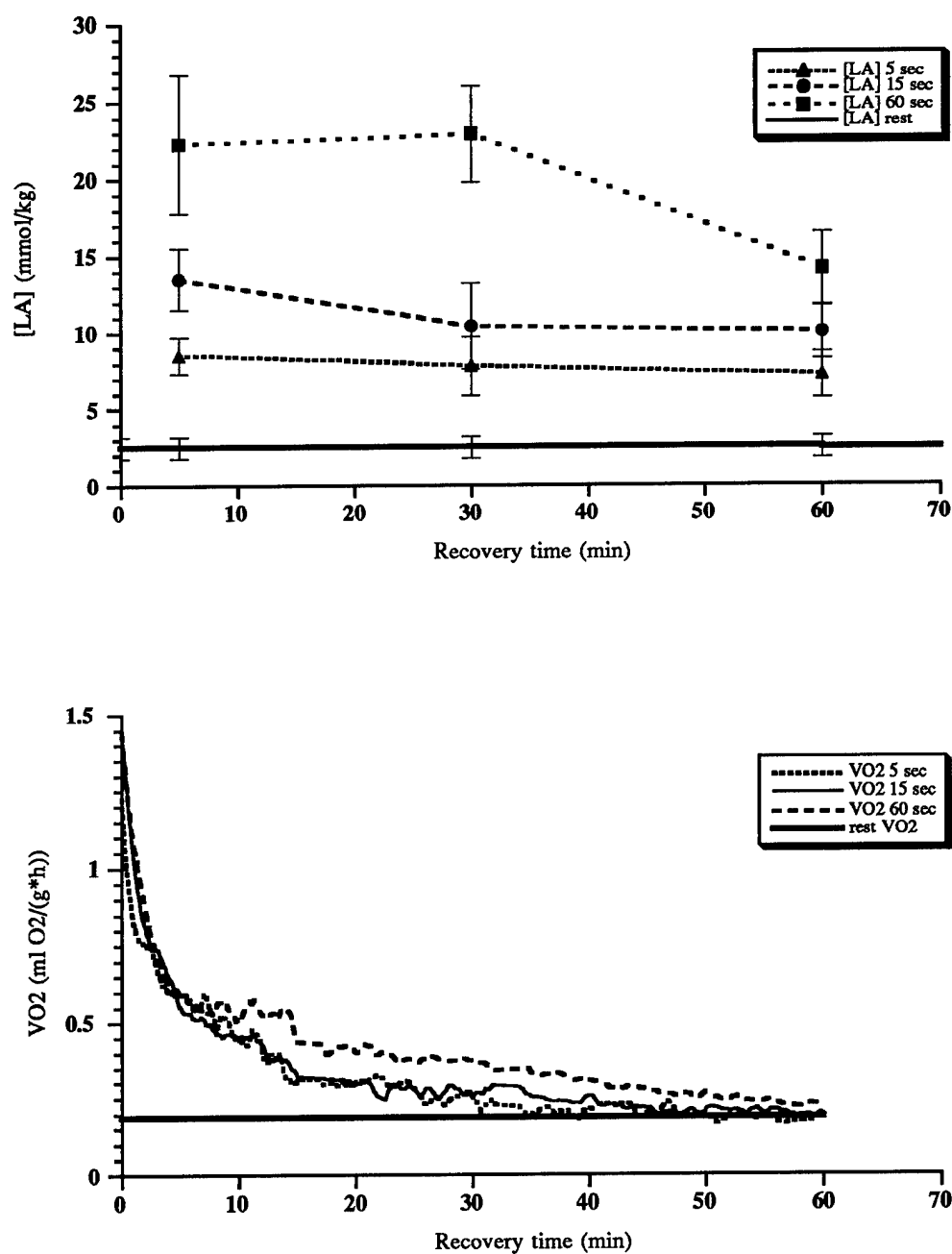


Figure 7. Blood lactate concentrations (mmol/kg) and oxygen consumption (ml  $O_2/(g \cdot h)$ ) during recovery following 5, 15, and 60 second sprints. For [LA], means are plotted  $\pm$  SEM ( $n=6$ ). For oxygen consumption, means of 6 animals are plotted.

statistically lower after 60 minutes of recovery compared to 5 minutes of recovery ( $p=0.684$  and  $0.458$ , respectively, student's paired t-test). Following the 60 second sprints, blood lactate concentration after 30 minutes of recovery after was not statistically lower than after 5 minutes of recovery. However, after 60 minutes of recovery following the 60 second sprints, a significant amount of blood lactate had been removed compared to 5 minutes of recovery ( $14.1 \pm 2.4$  mmol/kg and  $22.3$  mmol/kg respectively, student's paired t-test,  $p=0.005$ ).

### Discussion

The resting values of blood lactate are similar to those reported previously in the literature for this species of lizard: Bennett and Dawson, (1972)  $2.78$  mmol/kg; Gleeson and Dalessio, (1989)  $1.8 \pm 0.53$  mmol/kg; Wagner and Gleeson, (1996)  $1.6 \pm 0.34$  mmol/kg. Also, the peak levels of blood lactate observed after a 60 second sprint were similar to values of blood lactate following longer duration of exhaustive exercise for this animal (Bennett and Dawson, 1972; Gleeson and Dalessio, 1989; Wagner and Gleeson, 1996). I expected to see similar maximal lactate concentrations following 1 minute of exercise when compared to longer bouts of exercise because a majority of lactate accumulation in this reptile occurs during the first 30 seconds of exercise (Gleeson and Dalessio, 1989). Bennett and Dawson (1972) also reported that the highest lactate values after exercise occurred after exercise at  $40^{\circ}\text{C}$  which was the temperature used for my experiments.

Bennett (1982) reported that many lizards used anaerobic metabolism as an important energy yielding pathway to sustain short-term activity and that this anaerobiosis would cause the production of lactate by the exercising muscles. Therefore, it is not surprising to see such elevated levels of lactate following sprint activity in *D. dorsalis*. The accumulation of lactate in this iguanid lizard implies that it is using anaerobiosis to supplant its muscular energy demands during brief sprint

behavior. Lactate accumulations also suggest that the sprint speeds used by my specimens during these experiments were above the maximal aerobic speed. Therefore, these animals are operating above their aerobic threshold. Increased lactate concentrations are reported in other animals following exercise (humans, Brooks and Gaesser, 1980; fish, Milligan and McDonald, 1988; amphibians, Bennett and Licht, 1973, Gatten, Jr., *et al.*, 1992; and other reptiles, Bennett and Licht, 1972). Therefore, lactate production during exercise is a widespread phenomenon in the vertebrates.

The disposal of lactate following exercise was originally thought to be a driving force behind the elevation of oxygen consumption during recovery (Hill and Lupton, 1923). However, the oxygen debt hypothesis is too simplistic to adequately describe excess post-exercise oxygen consumption. From this experiment and other studies mentioned earlier, it is clear that plasma lactate concentrations are not correlated well with excess post-exercise oxygen consumption (Bennett and Licht, 1973; Gratz and Hutchinson, 1977; Hutchinson *et al.*, 1977; Bennett, 1978; Gleeson, 1980; Milligan and McDonald, 1988; Withers *et al.*, 1988b; Wagner and Gleeson, 1996. For example, Wagner and Gleeson (1996) found that  $\dot{V}O_2$  returned to resting levels prior to any significant removal of lactate in *D. dorsalis* following 5 minutes of exhaustive treadmill exercise. In fact, the complete removal of lactate required 3 hours for *D. dorsalis* (Gleeson and Dalessio, 1989; Wagner and Gleeson, 1996). In an earlier study, Bennett and Licht (1973), found a temporal disjunction between lactate removal and oxygen consumption in two species of amphibians. The trend of oxygen consumption returning to resting levels before significant amounts of lactate have been removed is also found in fish (Milligan and McDonald, 1988), mammals (Kayne and Alpert, 1964; Harris *et al.*, 1968), amphibians, (Withers *et al.*, 1988b), and serpentine reptiles (Gratz and Hutchinson, 1977; Hutchinson *et al.*, 1977). Brooks and Gaesser (1980) found the opposite phenomenon in rats where lactate removal was complete well

before oxygen consumption returned to resting levels. Despite being different from other animals, the recovery pattern of rats still exhibits a temporal disjunction between post-exercise oxygen consumption and lactate removal.

Gleeson and Dalessio (1989) found that following 5 minutes of exhaustive treadmill exercise, *D. dorsalis* converted 50% of its post-exercise lactate pool into glycogen and glucose. Only 16% of the lactate was oxidized during the first two hours of recovery (Gleeson and Dalessio, 1989). The oxidation of lactate is postulated to provides energy for the conversion of lactate back into glycogen (Margaria *et al.* 1933; Gaesser and Brooks, 1980). Gleeson and Dalessio (1989) reported that lactate oxidation represented 40% of the EPOC in *D. dorsalis* following 5 minutes of exhaustive treadmill exercise. In my study, only after 1 hour of recovery was a significant portion of lactate removed in these animals following 60 seconds sprints. However, lactate levels still remained above resting levels during this recovery period. Therefore, only a small amount lactate oxidation and/or reconversion to glycogen is probably taking place during the initial 60 minutes of recovery following 60 second sprints. Thus, lactate oxidation or reconversion into glycogen could account for a small portion of the EPOC seen in *D. dorsalis* following short sprints, but may not account for a large enough portion to have direct correlative effects. For example, following the shorter sprints of 5 and 15 seconds, there was no significant removal of lactate during 60 minutes of recovery. This may be attributed to the lower accumulations of lactate following the shorter bouts of exercise. The concentration of blood lactate may have effects on the removal rates of lactate. Therefore, lower concentrations of lactate may cause lower removal rates.

Perhaps the increased levels of lactate are having other physiological effects in these lizards. Increase rates of triglyceride-fatty acid (TG-FA) substrate cycling has been proposed as a possible contributor to EPOC (Gaesser and Brooks, 1984; Bahr, 1992). Perhaps the elevated lactate concentrations following exercise are affecting the

cycling rate of TG-FA substrates through an unknown mechanism. For these short bursts of activity there may be another important factor that has much greater effects on EPOC than lactate concentrations. Gleeson *et al.* (1993) found that the catecholamines were elevated in *D. dorsalis* following exhaustive treadmill exercise. Perhaps catecholamines are having substantial effects on EPOC following short sprints of activity.

## CHAPTER 5:

### THE EFFECTS OF THE CATECHOLAMINES ON EPOC

#### Introduction

The adrenal hormones, particularly the catecholamines, epinephrine and norepinephrine, are involved in the "fight or flight" response to stress seen in nature (Withers, 1992; Schreibman *et al.*, 1993;). The catecholamines operate via cellular  $\alpha$  and  $\beta$  cellular membrane receptors. Norepinephrine selectively binds  $\alpha$  receptors better than  $\beta$  receptors while epinephrine binds either receptor (Schreibman *et al.*, 1993). The actions of the catecholamines are activated via a g-protein second messenger system. The g-protein activates the cyclic adenosine monophosphate (cAMP)-adenylate cyclase pathway to mediate effects within the cell. The catecholamines have a variety of physiological effects regulated via this and other pathways.

The catecholamines have been shown to stimulate glycogenolysis in carp (Mazeaud, 1964), goldfish (Birnbaum *et al.*, 1976), trout (Mommensen *et al.*, 1988), rockfish (Danulat and Mommensen, 1990), rats (Larkin *et al.*, 1994), *D. dorsalis*, (Gleeson *et al.*, 1993), alligators (Coulson and Hernandez, 1983), turtles (Keiver and Hochachka, 1991), and dogs (Fujiwara *et al.*, 1996). Gluconeogenesis is also stimulated by the catecholamines (Coulson and Hernandez, 1983; Mommensen *et al.*, 1988; Danulat and Mommensen, 1990; Gleeson *et al.*, 1993). These hormones have been shown to increase the rates of TG-FA substrate cycling (Bahr, 1992) and to reduce blood flow and cause vasoconstriction in the extremities following exercise in humans

(Gullestad *et al.*, 1993) and marsupials (Ye *et al.*, 1995). Epinephrine has also been reported to increase heart rate as well (Bahr, 1992).

Increases in these hormones during and following exercise is widespread among the vertebrates: dogs (Cain, 1971, Cain *et al.* 1981), humans, (Bahr, 1992; Chmura *et al.*, 1994), reptiles (Coulson and Hernandez, 1983; Gleeson *et al.*, 1993), amphibians (Withers *et al.*, 1988a), and marsupials (Ye *et al.*, 1995). The effects of the catecholamines on metabolic rate and EPOC have been studied in numerous organisms. Barnard and Foss (1969) found that after administration of the  $\beta$ -blocker, propranolol, in mongrel dogs, arterial lactate was reduced by nearly 29% while mean EPOC was reduced 36.8% following 19 minutes of treadmill running. Cain (1971) found similar reductions in EPOC in dogs with  $\beta$ -blocker administration following exercise at low and high altitudes.  $\beta$ -adrenergic blockage reduced EPOC following 60 minutes of cycle ergometric exercise in humans (Børsheim *et al.*, 1994). These results seem to indicate a stimulatory effect of the catecholamines on EPOC through  $\beta$ -adrenergic stimulation. As stated earlier, Gladden *et al.* (1982) found that norepinephrine increased EPOC in isolated canine muscle preparations following 10 minutes of contractile activity. Gupta and Thapliyal (1985) found that epinephrine and norepinephrine both stimulated the rate of oxygen consumption of the whole body as well as the tissues (liver and skeletal muscle) in the garden lizard, *Calotes versicolor*.. It appears that the catecholamines, through  $\beta$ , and possibly  $\alpha$ -adrenoreceptor stimulation are having profound effects on EPOC. The purpose of this experiment was to try to isolate the effects of these hormones on EPOC following short burst, locomotion in the desert iguana.

### Materials and Methods

#### Animal care



Animal care was the same as described in Chapter 3.

#### $\alpha$ and $\beta$ blocker injections

For these experiments, the lizard was placed in a small polyvinyl chloride (PVC), tube that simulated a burrow. The tube was placed on the surface of the treadmill which was temperature regulated ( $40 \pm 1.0^\circ\text{C}$ ). Outside air was drawn across the animal through the tube and then sampled by the gas analyzers as described above (see Chapter 3). Gas analyzers were calibrated with sample gases as mentioned above. The animal was allowed to adjust to the artificial burrow for 2.5 hours. The data acquisition program was started 18 minutes before the end of the future exercise bout to acquire a baseline value of oxygen and carbon dioxide consumption and to monitor the activity of the lizard before the exercise bout. Five minutes prior to the dash, the animal was injected with either 14.3 or 143 mg/g body weight propranolol (Sigma P0884) (a  $\beta$ -blocker), 11.1 or 111 mg/g body weight phentolamine (Sigma P7547) (a general  $\alpha$ -blocker), or saline solution (Mora *et al.*, 1983). These chemicals are known to bind to the  $\alpha$  and  $\beta$  receptors which would prevent the catecholamines from binding to these receptors. After the injection, the animal was placed back in the artificial burrow. Five minutes post-injection, the animal was taken from the artificial burrow and induced to sprint for 5, 15, 30, and 60 seconds on the treadmill. The high dosages were used to determine the effectiveness of the lower dose at blocking the actions of the catecholamines. Immediately after the sprint, the animal was placed back into the artificial burrow and allowed to recover quietly for 79 minutes.

#### Results

Injection of propranolol and phentolamine both significantly shortened EPOC duration for most exercise regimens (Table 7, Fig. 8). The single exception was the 30 second sprint duration where the phentolamine treatment was not significantly lower than the saline control. EPOC was shortened by an average of 26% by propranolol

Table 7. Effect of Phentolamine and Propranolol injection on the duration of EPOC.

Exercise Duration	Treatment		
	Saline	Phentolamine (low)	Propranolol (low)
5	29.7±1.1 <b>a</b>	25.1±0.9 <b>b</b>	22.1±1.2 <b>b</b>
15	38.1±1.2 <b>a</b>	32.0±1.1 <b>b</b>	28.2±1.0 <b>b</b>
30	46.1±2.3 <b>a</b>	38.5±2.1 <b>a,b</b>	34.0±1.1 <b>b</b>
60	65.5±1.7 <b>a</b>	54.0±1.0 <b>b</b>	49.6±1.4 <b>b</b>

Values are mean  $\pm$  1 SEM (n=8) duration of EPOC (min). Exercise duration has the units, seconds. Values within the same row with different superscript letters are significantly different (students t-test,  $P < 0.008512$ ).

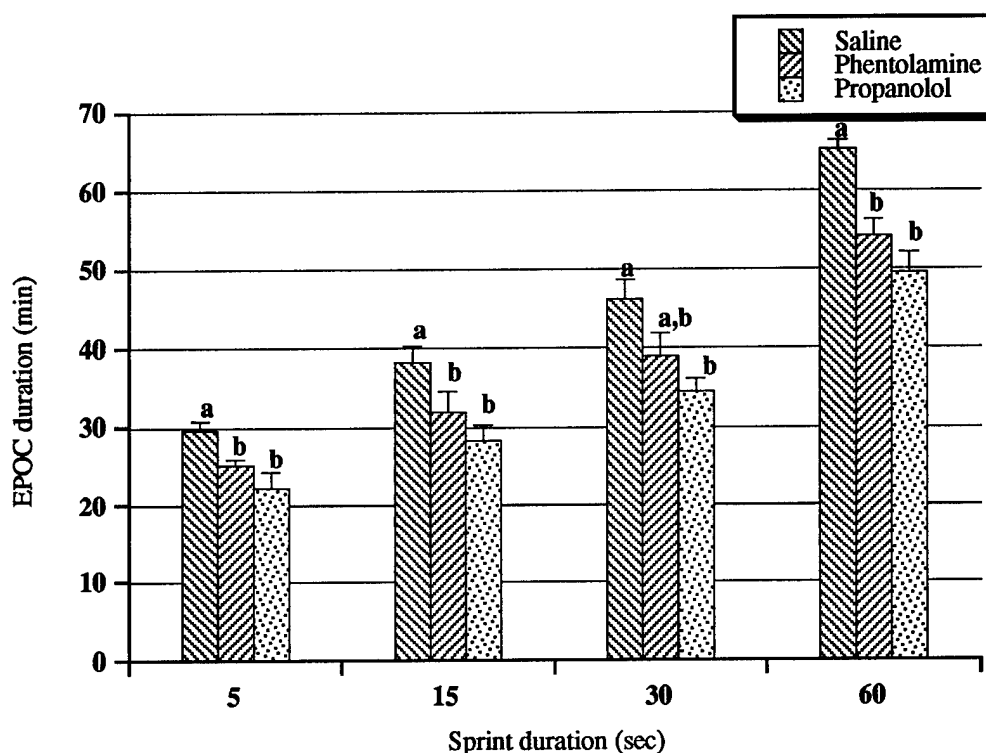


Figure 8. The effect of saline, propranolol, and phentolamine treatment on EPOC duration for all 4 exercise regimens. Low dosage treatments are shown. Y values (ml  $O_2$ /g) are means  $\pm$  1 SEM (n=8). Values within the sprint category with different superscript letters are significantly different (students t-test,  $P < 0.008512$ ).

injection and 16% phentolamine injection. There were no statistical differences in EPOC duration between phentolamine and propranolol treatments.

The volume of EPOC was also significantly effected by treatment (Table 8, Fig. 9). Following the 5 second sprints, propranolol injection reduced EPOC volume to 0.066 ml O<sub>2</sub>/g, compared to saline treatment, 0.11 ml O<sub>2</sub>/g. Phentolamine treatment

Table 8. Effect of Phentolamine and Propranolol injection on the volume of oxygen consumed during EPOC.

Exercise Duration	Treatment		
	Saline	Phentolamine (low)	Propranolol (low)
5	0.11±0.007 <b>a</b>	0.078±0.006 <b>b</b>	0.066±0.005 <b>b</b>
15	0.141±0.011 <b>a</b>	0.112±0.009 <b>b</b>	0.098±0.006 <b>b</b>
30	0.187±0.010 <b>a</b>	0.174±0.005 <b>a,b</b>	0.145±0.006 <b>b</b>
60	0.224±0.011 <b>a</b>	0.191±0.004 <b>b</b>	0.172±0.007 <b>b</b>

Values are mean ± 1 SEM (n=8) net volume of oxygen consumed during EPOC (ml O<sub>2</sub>/g). Exercise duration has the units, seconds. Values within the same row with different superscript **letters** are significantly different (students t-test, P<0.008512).

(0.174 ml O<sub>2</sub>/g) did not significantly alter the EPOC volume below the saline treatment (0.187ml O<sub>2</sub>/g) following 30 second sprints. However, the propranolol treatment volume (0.145 ml O<sub>2</sub>/g) was significantly lower than the saline treatment for this sprint duration. The average EPOC volume reduction pooled from all exercise regimens was 18% for the phentolamine and 28.8% for the propranolol treatment.

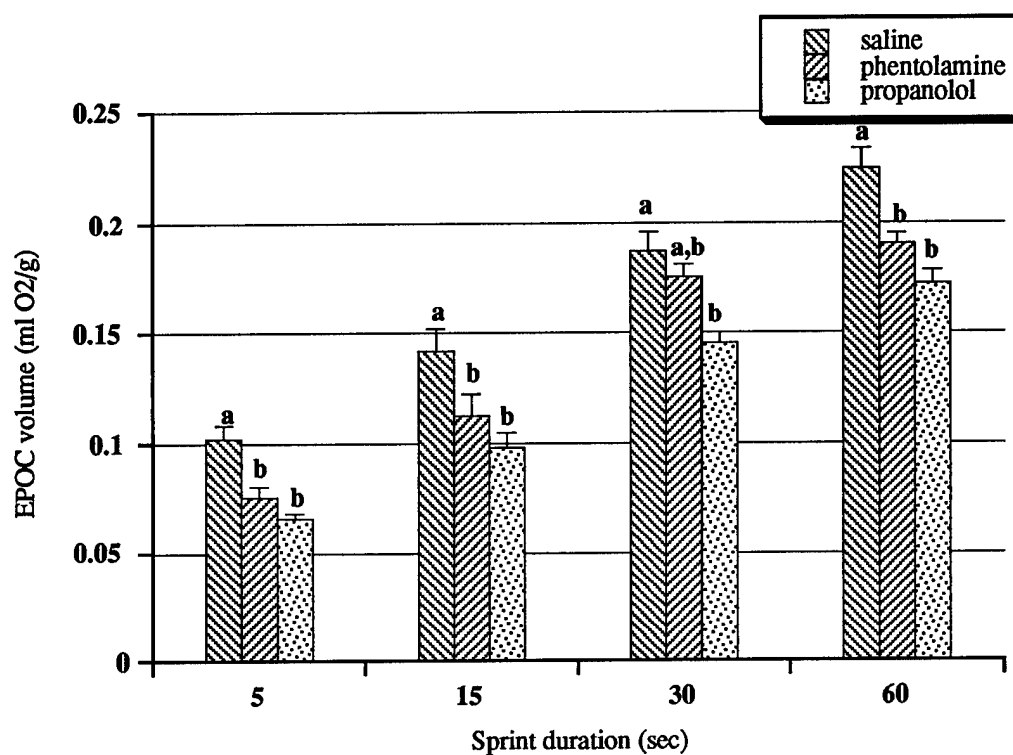


Figure 9. The effect of saline, propranolol, and phentolamine treatment on EPOC volume for all 4 exercise regimens. Low dosage treatments are shown. Y values (ml O<sub>2</sub>/g) are means  $\pm$  1 SEM (n=8). Values within the sprint category with different superscript letters are significantly different (students t-test,  $P < 0.008512$ ).

There were no statistical differences in the net volume of EPOC between phentolamine and propranolol treatments, nor between the low and high dosages of propranolol and phentolamine (Table 9) (Student's t-test,  $P=0.2648$ ).

Table 9. Effect of high and low dosages of Phentolamine and Propranolol injection on the net volume of EPOC.

Exercise Duration	Treatment	
	Phentolamine (high)	Phentolamine (low)
5	$0.072 \pm 0.007$	$0.078 \pm 0.006$
15	$0.101 \pm 0.011$	$0.112 \pm 0.009$
30	$0.181 \pm 0.010$	$0.174 \pm 0.005$
60	$0.184 \pm 0.011$	$0.191 \pm 0.004$

Exercise Duration	Treatment	
	Propranolol (high)	Propranolol (low)
5	$0.061 \pm 0.007$	$0.066 \pm 0.005$
15	$0.099 \pm 0.011$	$0.098 \pm 0.006$
30	$0.142 \pm 0.010$	$0.145 \pm 0.006$
60	$0.183 \pm 0.011$	$0.172 \pm 0.007$

Values are mean  $\pm$  1 SEM (n=8) net volume of oxygen consumed during EPOC (ml  $O_2/(g \cdot h)$ ). Exercise duration has the units, seconds.

### Discussion

The reduction in EPOC seen in this experiment following  $\alpha$  and  $\beta$ -blockage agrees well with previous studies done on dogs (Barnard and Foss, 1969) and humans (Børshheim, 1994). These reductions in EPOC via the injection of catecholamine antagonists suggests that these hormones have a stimulatory effect on EPOC. Both  $\alpha$  and  $\beta$  adrenergic stimulation seem to play an important role in EPOC. These results agree with the findings of other authors (Barnard and Foss, 1969; Cain, 1971; Gladden *et al.*, 1982). These hormones may be administering their effects on EPOC via a number of mechanisms.

Gupta and Thapliyal (1985) found both epinephrine and norepinephrine to stimulate oxidative metabolism in *Calotes versicolor*. Coulson and Hernandez (1983) reported that the catecholamines stimulated oxygen consumption in the American alligator. Gleeson *et al.* (1993) reported that epinephrine stimulated both lactate oxidation and lactate carbon incorporation into glycogen in *D. dorsalis* following exhaustive exercise. Catecholamines can also stimulate glycogenolysis and gluconeogenesis in fish, mammals, and lizards (Mazeaud, 1964; Birnbaum *et al.*, 1976; Coulson and Hernandez, 1983; Kraus-Friedman, 1984; Mommsen *et al.*, 1988; Danulat and Mommsen, 1990; Keiver and Hochachka, 1991; Gleeson *et al.*, 1993; Larkin *et al.*, 1994; Fujiwara *et al.*, 1996). The stimulatory effects of the catecholamines on glycogenolysis and gluconeogenesis may explain some of these hormone effects on excess post-exercise consumption. If the *in vivo* rates of glycogenolysis and gluconeogenesis were slowed by catecholamine blockage, the energy expenditure of the animal might be reduced. The reduction in energy expenditure could then serve to reduce EPOC as well. Gaesser and Brooks (1984) reported that the portion of energy gained via the oxidation of lactate after exercise is used to provide ATP for the reconversion of glycogen from lactate. Gleeson and Dalessio (1989) reported that *D. dorsalis* selectively utilizes lactate over glucose as a gluconeogenic substrate following 5 minutes of exhaustive treadmill exercise. Also, this lizard converts a majority of its lactate to glycogen after exercise (Gleeson and Dalessio, 1989). If the injections of propranolol and phentolamine are slowing the rate of gluconeogenesis, then perhaps both EPOC duration and volume would be reduced. Also a reduced rate of glycogenolysis may limit the amount of glycogen available for energy conversion and could reduce EPOC.

Bahr (1992) reported that increased levels of TG-FA substrate cycling can contribute to EPOC substantially (approximately 50%). Børsheim *et al.* (1994) reported that both epinephrine and norepinephrine stimulate the rates of TG-FA cycling

in humans. The energy cost of increase cycling rates could serve to elevate oxygen consumption and prolonged EPOC. If the catecholamine's actions on the TG-FA cycle are blocked by the antagonistic drugs, then EPOC may be reduced as well.

The catecholamines have also been reported to decrease blood flow and cause vasoconstriction in humans (Gullestad *et al.*, 1993) and marsupials (Ye *et al.*, 1995). If blood flow is restricted to the muscles of *D. dorsalis* following adrenergic blockage, then the rates of gluconeogenesis and lactate oxidation might also be reduced which could lower the energy demands of this organism. The lowered energy demands would then cause a reduction in EPOC. Gleeson *et al.* (1993) reported that catecholamines increase in *D. dorsalis* following exhaustive exercise. Epinephrine levels remained elevated 2 hours after exercise while norepinephrine levels had returned to rest by 2 hours of recovery (Gleeson *et al.* 1993). Withers *et al.* (1988a) reported that catecholamines were elevated in *Bufo marinus* following exercise. Elevated catecholamines have also been reported in man (Chmura *et al.*, 1994) and dogs (Cain, 1971). It appears that the stress response to exercise itself may have important implications on EPOC as well as vertebrate energetics.

The very act of exercising causes catecholamine levels to increase. This has been termed the "fight or flight" response in animals. The mobilization of glycogen associated with this response may account for some of EPOC. Another consequence of this response may be a heightened "alertness" of the animal. This proposed heightened alertness could cause the animal to maintain a more alert posture which would increase the animal's metabolic rate. The alertness posture of the animal could be related to an increase in heart rate. Increased heart rate following exercise are thought to be responsible for a portion of EPOC (Gaesser and Brooks, 1984; Bahr, 1992). Furthermore, Bahr (1992) stated that epinephrine could cause an increase in heart rate. If epinephrine is stimulating the heart rate of *D. dorsalis* following exercise, then recovery oxygen consumption may be elevated and cause a portion EPOC. A reduction

in heart rate caused by catecholamine blockage could reduce recovery oxygen consumption. Despite the reductions in EPOC duration and volume seen following adrenoceptor blockage, *D. dorsalis* continued to display a prolonged EPOC following sprint activity. EPOC was only reduced by approximately one third following  $\beta$ -adrenergic blockage and approximately one fourth following  $\alpha$ -blocker injection. Thus, there are other mechanisms that are affecting EPOC other than a response to catecholamines. The other possible causes of a prolonged EPOC in *D. dorsalis* following short-term exercise will be discussed in chapter 7.



## CHAPTER 6

### THE ENERGETIC COST OF NON-SUSTAINABLE LOCOMOTION

#### Introduction

The energetic cost of terrestrial locomotion or the cost of transport,  $C_t$ , has traditionally been measured in terms of long duration, sustainable types of locomotion (Taylor, 1973; Heglund and Taylor, 1982; John-Alder *et al.*, 1986). Extended duration locomotion allowed these authors to measure the steady-state levels of oxygen consumption achieved by the animals at any given velocity. These authors plotted steady-state  $\dot{V}O_2$  versus running velocity to calculate the cost of transport from the slope of this relationship (Taylor, 1973; Heglund and Taylor, 1982; John-Alder *et al.*, 1986). The slope of this line was termed the minimum cost of locomotion and was achieved at the animal's maximum speed. The work of these authors found that as mass increases, the mass specific cost of transport decreases (Taylor, 1973; Taylor *et al.*, 1982; John-Alder *et al.*, 1986; Heglund and Taylor, 1988). The cost of transport was also related to speed in that the faster an animal ran, the lower the mass-specific cost of transport was (Taylor, 1973; Heglund and Taylor, 1982; John-Alder *et al.*, 1986). At an animal's maximum speed, the cost of transport was reduced to the lowest value for that animal. Few studies have looked at the cost of very short bursts of activity. Many animals, particularly the lower vertebrates, rely heavily on short bursts of activity. Few animals, except migrators, such as caribou and wildebeest, undergo the duration of locomotion used in the traditional experiments. The purpose of this final chapter will be to look at the energetic cost of quick bursts of activity. The cost of

non-sustainable locomotion, ( $C_{ns}$ ), based upon terminology offered by Wager and Gleeson (1996), is a useful quantification of the energetic costs, in terms of oxygen consumption, of non-sustainable locomotion. The cost of non-sustainable locomotion differs from the traditional estimates of the cost of locomotion in that  $C_{ns}$  includes the net volume of oxygen consumed both during and after exercise (EPOC).

### Materials and methods

Data reported in Chapter 3 were used in this analysis of  $C_{ns}$  and the immediate cost of transport,  $IC_t$ . I have used the term "immediate cost of transport" to distinguish it from the traditional cost of transport because the calculation of these two variable differs. To calculate  $IC_t$ , I took the volume of oxygen consumed during the bout of exercise above resting levels (volume A, from Fig. 1) and divided that value by the distance traveled during the sprint. This results in a value analogous to the traditional cost of transport and has the units  $ml\ O_2 \cdot g^{-1} m^{-1}$ ). For the calculation of  $C_{ns}$ , I took the entire net volume of oxygen consumed during and after the sprint above resting levels (volume A + volume B, Fig. 1) and divided that value by the distance traveled. A predictive value of the traditional cost of transport for a 30g ectothermic lizard, ( $0.0016\ ml\ O_2 \cdot g^{-1} m^{-1}$ ) was found by using the equation of John-Alder *et al.* (1986) (equation 2).

### Results

The values of  $IC_t$  following short sprints are substantially lower than the predicted value of  $C_t$  from equation 2 for a 30g animal. There was a positive relationship between sprint duration and the  $IC_t$  (Fig. 10). The 5 second sprints generated an  $IC_t$  of  $0.00047\ ml\ O_2 \cdot g^{-1} m^{-1}$ ) while 60 seconds sprints generated a value

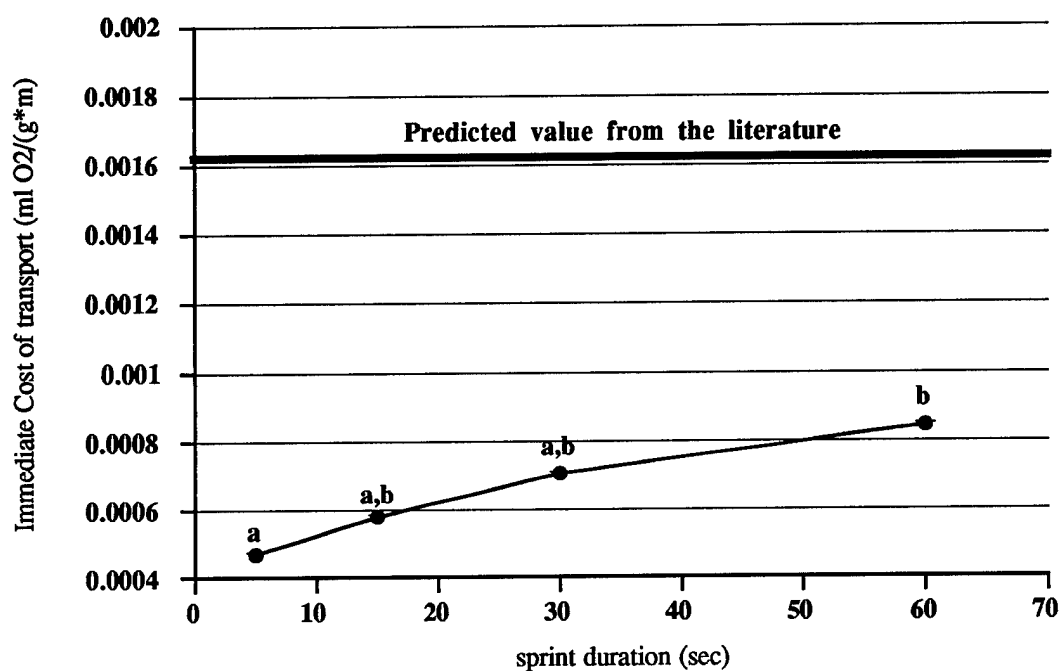


Figure 10. The relationship between the immediate cost of transport and exercise duration for *D. dorsalis*. Means of 11 animals are plotted  $\pm 1$  SEM. The predicted value of Ct was calculated using equation 2 and a mass of 30g. Points with different superscripts are statistically different.

that was 55% higher, ( $0.00084 \text{ ml O}_2\cdot\text{g}^{-1}\text{m}^{-1}$ ). The best fit linear equation fit to these data predict that  $\text{IC}_t$  will intercept the predicted value from the literature, ( $0.0016 \text{ ml O}_2\cdot\text{g}^{-1}\text{m}^{-1}$ ), at a sprint duration of 2.8 minutes.

Conversely, the calculations of the  $\text{C}_{\text{ns}}$  are considerably higher than the predicted value (Fig. 11). These values show an inverse relationship between  $\text{C}_{\text{ns}}$  and exercise duration. The 5 second value of  $\text{C}_{\text{ns}}$  ( $0.04 \text{ ml O}_2\cdot\text{g}^{-1}\text{m}^{-1}$ ) is greater than 25x the predicted value ( $0.0016 \text{ ml O}_2\cdot\text{g}^{-1}\text{m}^{-1}$ ) and 4x the 60 second value ( $0.01 \text{ ml O}_2\cdot\text{g}^{-1}\text{m}^{-1}$ ). Even the lowest value of  $\text{C}_{\text{ns}}$ , achieved during the 60 seconds sprints, is greater than 6x the predicted value. It appears that these values asymptotically approaching the predicted line (Fig. 11). A best fit equation for the cost of non-sustainable data predicts that this line would intersect the predicted value at a sprint duration of 2.5 minutes.

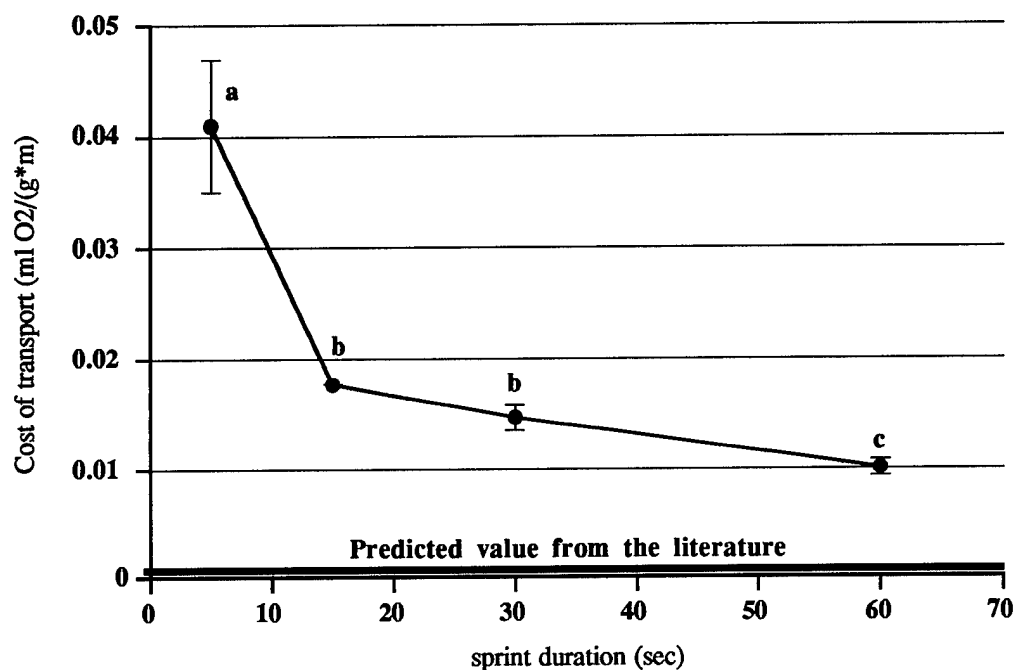


Figure 11. The relationship between the cost of transport and exercise duration for *D. dorsalis*. Means of 11 animals are plotted  $\pm 1$  SEM. The predicted value of Ct was calculated using equation 2 and a mass of 30g. Points with different superscripts are statistically different.

## Discussion

I compared the immediate cost of transport to the traditional cost of transport from the literature. Although a direct comparison of these two variables may be limited in value because they are derived using different methods, the equations of Taylor *et al.* (1982) and John-Alder *et al.* (1986) are the only comparisons available in the literature. I found the immediate cost of transport for these short sprints in *D. dorsalis* is lower than the predicted value obtained from the literature. The values of  $IC_t$  obtained in this experiment should be lower than the predicted value because the predicted value is based on steady-state measurements of oxygen consumption. The extremely short sprints tested in my experiment do not allow the lizard to achieve a steady-state  $\dot{V}O_2$ . Because of the lag time in oxygen consumption seen at the beginning of exercise, oxygen consumption cannot achieve the level appropriate for that intensity of exercise. Therefore, the oxygen consumed during a 5 second sprint for a given speed is less than the volume of oxygen consumed during 5 seconds of steady-state activity at the same speed. For these reasons, I wanted to investigate the relationship between the traditional cost of transport and the cost of non-sustainable locomotion.

Because EPOC represents a large consequential volume of oxygen following exercise, its inclusion into the cost of non-sustainable transport greatly increases the cost of non-sustainable transport over the predicted value. I found that EPOC was a very energetically costly event. EPOC is energetically important to animals undergoing short term exercise, because its volume represents a much greater portion of the whole than the volume consumed during exercise. Perhaps, the true ecological cost of transport (Garland, Jr., 1984) has previously been underestimated because the EPOC volume was not included as an energetic cost in most earlier studies. Therefore, I propose that EPOC volume should be included in a calculation of the cost of non-sustainable transport. To illustrate the significant effect of EPOC on the energetic costs of locomotion in animals I will offer a hypothetical scenario using *D. dorsalis*.

In this scenario, the animal sprints 3 different times during a 3 hour time period. The first sprint is 5 seconds, followed by a 15 second and another 5 second sprint. The total distance traveled during the sprints is 14 meters. By multiplying this value with the predicted value from equation 2 ( $0.0016 \text{ ml O}_2/(\text{g}\cdot\text{m})$ ), we find a predictive value of  $0.022 \text{ ml O}_2/\text{g}$  consumed during this locomotion. Using data from my experiments one would find that the volume consumed during and after these three hypothetical bouts of exercise would be  $0.424 \text{ ml O}_2/\text{g}$ . This value is 19x greater than the traditionally derived value. Therefore, if an ecologist was trying to generate an energy budget based on traditional estimates of the cost of locomotion, they would greatly underestimate the true amount of oxygen consumed during and after the bouts of activity. Kenagy and Hoyt (1989) have found that ground squirrels travel in short sprints from one location to another during the day. The oxygen consumed by these squirrels during the actual locomotion is probably quite small compared to the consequential EPOC volume. Therefore, the ecological cost of transport (ECT) proposed by Garland, Jr., (1984) may be underestimated if only the traditional measurements are used. The ECT of many animals has been predicted to account for only a small percentage of the total daily energy expenditure (DEE) of an animal (Garland, Jr., 1984). Therefore, the cost of locomotion has been given little significance to the energy budgets of animals. If EPOC were included in the ECT, it may drastically increase the ECT and it would account for a larger portion of the daily energy expenditure. It is possible that the cost of non-sustainable locomotion accounts for a much greater percentage of the total daily energy expenditure once the energetic consequences of EPOC are included.

To avoid confusion with the traditional cost of transport and to account for the differences between the traditional measurement protocol and my experimental protocol, another term could be defined (i.e. the cost of activity) that would include not only the consequences of exercise, but also the entire suite of physiological factors that

occur during and after a burst of activity (i.e. stress response). This term could then be used to generate another term, the ecological cost of activity, which would be separated from the traditional ecological cost of transport, in that the energetic cost incurred during EPOC would be included in the ecological cost of activity value. I contend that the ecological cost of activity may be much more important to the daily energy expenditure than the traditional ECT value.

Another interesting finding of these experiments is that the linear equations fit to the  $IC_t$  and  $C_{ns}$  data intercept the predicted cost of locomotion from John-Alder *et al.* (1986) at approximately the same time (2.7 minutes). From my best fit linear equation relating EPOC duration to sprint duration (equation 10) I predicted a value of EPOC duration following 5 minutes of sprint activity which was significantly higher than the actual EPOC measured by Wagner and Gleeson (1996). Wagner and Gleeson (1996) found an EPOC of approximately 90-120 minutes following 5 minutes of activity. Using equation 10, I solved for the sprint duration that would yield a recovery time of 105 minutes (halfway between 90 and 120 minutes). Using this approach I generated a sprint time of 2.3 minutes which is close to the predicted values found from the cost of locomotion intercept data. Perhaps equation 10 is not valid for 5 minutes of exercise because five minutes of exercise is simply too long to fit with the findings of shorter duration exercise. The  $IC_t$  and  $C_{ns}$  data intercepted the predicted value of the cost of locomotion at roughly 2.7 minutes. Therefore, I predict that only sprints of less than 2.7 minutes will follow the relationships found in my experiments. It may be that there is a threshold level reached after sprints of greater than 2.7 minutes. Additionally, because *D. dorsalis* cannot sustain sprint speeds for greater than approximately 2 minutes based on my observations and the results of John-Alder and Bennett (1981) the cut-off value of 2.7 minutes is valid.

Based on the cut-off value there appear to be differences in the energetic cost of short and long term exercise. Perhaps maximal levels of physiological EPOC factors



(i.e. phosphagen and glycogen depletion, catecholamine and lactate levels) are achieved after sprints of approximately 2.5 minutes in length. The animals may not be able to sustain sprint speeds after this time. Recalling my results from Chapter 4 we can see that blood lactate accumulation after 1 minute of sprint exercise was very similar to the blood lactate levels achieved after 5 minutes of exercise. Therefore, lactate concentrations probably reach a peak value after 1 minute of exercise. Additional attempts at sprinting beyond this 2.5 minute threshold may not elevate lactate and catecholamine levels further, nor decrease phosphagen and glycogen concentrations further. This would cause recovery time and the net volume of EPOC to reach plateau values.

## CHAPTER 7

### FINAL CONCLUSIONS AND AREAS OF FUTURE RESEARCH

My experiments have shown that following short sprints of activity, *Dipsosaurus dorsalis* exhibits a prolonged period of elevated oxygen consumption. Furthermore, I have shown that the slow intervals (2-6) of EPOC are effected by sprint duration while the first ten minutes of EPOC were unaffected by exercise duration. Hagberg *et al.* (1980) found similar results in that exercise duration had effects on the slow component of EPOC, but not the rapid component. The slow component used by Hagberg *et al.* (1980) was found by using a double exponent curve fit to their recovery data. This curve was then broken into two components, rapid and slow, by using the different time components of the double exponential equation. The effects of increasing exercise duration on EPOC may be attributed to a number of factors. Hagberg *et al.* (1980) attributed 60-70% of the slow component of EPOC to the  $Q_{10}$  effect. However, Gore and Withers (1990a) attributed only 11-36% of the 8-hour EPOC to the  $Q_{10}$  effect. Gore and Withers (1990a) further stated that their estimates of the effect of increased body temperature may have been underestimated. Body temperature was observed to increase in human subjects in these studies following exercise of varying duration and intensities (Hagberg *et al.*, 1980; Gore and Withers, 1990a) However, increased body temperature does not seem to be an important factor in my experiments because ectotherms do not exhibit any appreciable increase in body temperature during or following exercise (Withers, 1992).

Hagberg *et al.* (1980) also cited the differences in lactate concentrations following different exercise duration as a possible explanation for the differences in EPOC seen with varying exercise. However, the difference in lactate could only account for 30% of the difference in EPOC 5 and 20 minutes of exercise at 80% of  $\text{VO}_{2\text{max}}$  (Hagberg *et al.*, 1980). I have shown differences in blood lactate concentrations following different lengths of sprint activity. However, after the 5 and 15 second sprints, oxygen consumption had returned to resting levels within 60 minutes, yet a significant level of lactate had not been removed from the blood during the same time frame. Only after the 60 second sprints was a significant amount of lactate removed during 60 minutes of recovery. However, lactate levels at the 60 minute recovery point were still well elevated above resting levels. The differences in blood lactate concentrations do not have any significant effect on the duration or magnitude of EPOC because little lactate was removed during the oxygen consumptive recovery of these animals. Therefore, lactate removal does not appear to be an important factor contributing to EPOC in *D. dorsalis*.

Numerous authors have suggested that elevated catecholamine levels could be an important factor in EPOC (Hagberg *et al.*, 1980; Gaesser and Brooks, 1984; Bahr 1992). My data support this suggestion. Administration of catecholamine blockers caused significant reductions in both EPOC duration and volume. The gluconeogenic and glycogenolytic effects of these hormones may explain their effects on EPOC as well as their stimulatory effects on heart rate and the triglyceride-fatty acid cycle. Other potential causes of EPOC could be the oxygen cost of breathing, replenishment of myoglobin and hemoglobin oxygen stores, and cellular phosphagens (Gaesser and Brooks, 1984; Bahr, 1992). Further research should be conducted using other organisms to test the relationship between short term exercise and EPOC. Future experiments could also test the effect of simultaneous  $\alpha$  and  $\beta$  adrenergic receptor blockage to determine if the effects seen through individual receptor blockage are

additive or in conjunction with one another. Additional experiments could be performed to measure the effects of lactate concentrations and catecholamine levels on the rates of substrate cycling and heart rate and ventilation. The depletion of cellular levels of phosphagen and glycogen as well as O<sub>2</sub> depression of the myoglobin and hemoglobin could also be measured following variable duration of short term exercise.

These experiments have provided interesting results about the cost of transport for this species. The immediate cost of transport incurred during brief bursts of activity is lower than predicted by the literature for this animal. However, when the energetic cost of EPOC was included in the measurement of the cost of non-sustainable locomotion, this value greatly exceeded the traditional estimate. I propose that for short bursts of activity, EPOC volume should be included in the cost of non-sustainable locomotion. This value could be termed the cost of activity and would be very helpful in developing energy budgets for animals in the field. These types of experiments should be continued across the realm of animal species and types of locomotion. It is clear that the energetic costs incurred as a consequence of short, sprints of activity are different than the costs associated with long term activity. Similar experiments with animals of varying mass and locomotor activity should help to define the true energetic cost of short term sprints.

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